Book of Abstracts

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of Nanomaterials

8-9 Sept
2022

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Conexión Nanomedicina CSIC

Thursday, 8th of September

	Speaker	Title
9:15		**Registration at ICMM, Campus Cantoblanco**
9:45	SBAN Organizers	Presentation
		Chairperson: Fernando Herranz
10:00	**Oihane Ibarrola**	**Magnetic nanoparticles upscaling for early clinical phases multimodal cancer therapy**
		DRUG DELIVERY
10:30	Alejandro Postigo	DNA nanostructures customized for therapeutic delivery
10:45	Manel Estruch Blasco	Synthesis and characterization of hybrid nanomaterials for enhanced metallo prod-drugs delivery
10:50	Thais Fedatto Abelha	Silicon oxide microparticles with covalent and non-covalent immobilization of porphyrin present enhanced singlet oxygen generation and relevant cell uptake
10:55	Andrés Ramos-Valle	Accessible synthesis of silica DNA "nanofossils": applicability in gene storage/preservation and delivery
11:00	Eva Arroyo	Lipid nanocarriers for prevention of biofilm formation in cystic fibrosis patients
11:05	Laura Fernández-Méndez	Microfluidic synthesis of drug nanocarriers for crossing cellular and non-cellular biological barriers
11:10	IESMAT	IESMAT presentation
		Conference picture taken
11:20		**COFFEE BREAK + POSTER SESSION (odd numbers 1, 3, ...)**
		Chairperson: Helena Gavilán
12:15	**Eduardo Oliver**	**New diagnostic and therapeutic targets in murine models of cardiopulmonary disease**
12:45	Javier Idiago-López	Bioorthogonal click chemistry on living cell membranes: creating hotspots for transient increase of living cell membrane permeability
13:00	Urs O. Häfeli	Hyperbranched Polyglycerol Prodrug of Methotrexate is an Effective Nanomedicine for the Treatment of Rheumatoid Arthritis in a Preclinical Model
		NEURO
13:15	Esther Benayas	Exploring iron oxide nanoparticles for neural regeneration
13:20	Julia Martínez Ramírez	Approaching neural regeneration by using magnetic responsive hydrogels made of natural polymers
13:25	Beatriz L. Rodilla	Core@shell nanostructured electrodes for neural interfacing
13:30		**LUNCH BREAK + POSTER SESSION (even numbers 2, 4, ...)**
		Chairperson: Jesús Ovejero
14:30	**Verónica Salgueiriño**	**The key role of the surface topography and biofunctionalization of magnetic nanoparticles in biological media**
		SENSORS
15:00	Amalia Coro	Synthesis and characterization of semiconductor nanoparticles as luminescence nanothermometers in mice eyes
15:15	Irene Zabala Gutierrez	In-depth Study of Rare-earth Nanostructures to Design Efficient Luminescent Nanothermometers for Biological Applications
15:30	Manuel Gutiérrez-Capitán	Quantitative detection of SARS-CoV-2 RNA using a compact fluidic electrochemical biosensor platform
15:45	José Luis Marqués	Radio frequency impedance monitoring of inductive sensors for quantification of COVID-19 immunoresponse.
16:00		**COFFEE BREAK + POSTER SESSION (odd numbers 1, 3, ...)**
		Chairperson: Ana Espinosa
17:00	**Maria Mittelbrunn**	**How does time-dependent deterioration of T cells contribute to tissue senescence?**
		OTHER APPLICATIONS
17:30	Domingo Barber	Iron oxide and iron oxyhydroxide nanoparticles exert anti-viral activity in SARS-CoV-2-infected Vero E6 cells
17:45	Ana González-Paredes	Nanoparticles as a tool for management of biofilm-associated infections
18:00	Aitor Larrañaga	Modulating the layer-by-layer nanoarchitectonics via the incorporation of nanoencapsulated enzymes to perform enzymatic cascade reactions with potential biomedical application
18:15	Jose Antonio Laz-Ruiz	Hybrid-tag polymeric nanotrackers as universal reagents for long-term live-cell barcoding

Friday, 9th of September

	Speaker	Title
		Chairperson: Ana González Paredes
9:00	**Lourdes Basabe**	**Microchips for analysis of fluids and biological systems**
		CANCER
9:30	Nuria Lafuente-Gómez	A Magnetic Nanoparticle-Based Vaccine Generates Anti-Tumour Immunity in vitro and in vivo
9:45	Ana Márquez-López	Targeting Tumor Endothelial Marker 8 in metastatic disease
10:00	Catarina Coutinho	Visual and Multiplexed Detection of MicroRNAs Using Functionalized Gold Nanoparticles
10:15	Aritz Lafuente	MAPSULES: Multimodal theranostic agents for externally controlled and non-invasively monitored nanotherapies
10:30	Teresa Valero	Multifunctionalization strategies on nanoparticles for diagnostic chemical proteomic
10:35	Neus Daviu	Increasement of Oxidative Stress by IONPs alters mitochondrial bioenergetics and mitochondrial dynamics in cancer cells
10:40		**COFFEE BREAK + POSTER SESSION (even numbers 2, 4, ...)**
		Chairperson: Lucía Gutiérrez
11:30	**María Moros**	**Design of nanoactuators for tissue regeneration**
		HYPERTHERMIA
12:00	David Serantes	Inner-particle magnetization dynamics in relation to magnetic chaining/unchaining
12:15	Yilian Fernández-Afonso	Magnetic Nanoparticle transformations and the effect on their heating properties
12:30	Lorena García Hevia	Nanorod Photothermal Therapy Targeting Head and Neck Cancer Using a Modified Toxin Natural Ligand
12:45	David Egea-Benavente	Searching candidates for Magnetic Hyperthermia Therapy through shape and size rational design of MNPs
12:50	Rosalía López-Méndez	Development of multifunctional nanoplatforms based on highly stable iron oxide nanoparticles for therapeutic applications
12:55	M.Lázaro-Callejón	Non spherical magnetite nanoparticles for biomedical applications
13:00	**Mónica Carril**	**Fluorinated nanoparticles for 19F MRI and as reporters for protein corona**
13:30	**Puerto Morales**	**Closing remarks**

	First Author	Title
		DRUG DELIVERY
P1	Carlos Díaz-Ufano	Reproducible magnetic iron oxide nanocarriers by microwave assisted synthesis
P2	Raúl Gimeno Ferrero	Functionalitation of Iron Oxide NPs Using New PEG-Derived Ligads and Micelles Encapsulation
P3	Carlos Peris Torres	NANO3DEVICES: theranostic nanodevices with translational application.
		NEURO
P4	Ana Rodríguez Ramos	Raman spectroscopic assessment of Hsp90 amyloid refolding capacity
P5	Miguel Esteban Lucía	Neural Stem Cells Differentiation on Densely Packed High Aspect Ratio Nanopillars
P6	Paula Fernández	Development of Quantum Dot platform to measure pathological proteins and pharmacological action in lymphoblasts from ALS patients
		SENSORS
P8	Saman Bagherpour	BODIPY functionalized silicon oxide microparticles for intracellular glutathione sensing
P9	J.-Pablo Salvador	How nanobodies can improve current diagnosis tools and therapeutic approaches in lower respiratory tract infections.
P10	Ana Rodríguez-Galet	New molecular assay based on nanotechnology for the HIV P24 early detection
P11	Ana Valadés-Alcaraz	Characterization of aptamers for HIV-1 protease, integrase and p24 detection across HIV variants
P12	Mónica Dhanjani	Benign and versatile synthesis of iron oxide nanoparticles and their study in biomedical applications
P13	Elizabeth Champa	Fluorinated Smart Nanoprobes with Applications for Detection of MMP-2/9 after Stroke by 19F MRI
P14	Aitor Herraiz Pérez	IRON OXIDE NANOPARTICLES FOR T1 POSITIVE CONTRAST IN MAGNETIC RESONANCE IMAGING.
		CANCER
P15	Noelia Santander Acerete	Functionalization of T-cells with microwave synthesized magnetic nanoparticles for their magnetic retention
P16	Alberto Martín-Asensio	Tumor solid stress reduces the nanomedicines penetration in tumors
P17	César del Valle Pérez	Preparation of Fe3O4/Au nanostructures for biomedical applications
		HYPERTHERMIA
P18	Marina París	Biotransformed plasmonics of gold nanoparticles
P19	Nadia Pastor	Fe/Au/Cu nanostructures for biomedical applications
P20	Alejandro Casillas-Rubio	Rare-earth nanostructures as fluorescent nanothermometers and nanoheaters
P21	Oscar Iglesias	Taming the influence of dipolar interactions in nanoparticle assemblies for magnetic hyperthermia
		OTHER APPLICATIONS
P22	Nour al Hoda Al Bast	Opto Electrical Nano-reactors for Wireless Cell Stimulation
P23	Iban Llamas	Fabrication and characterization of BiTe-based NPs for Potential use as contrast agent in X-ray imaging

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DNA nanostructures customized for therapeutic delivery

Alejandro Postigo[1], Natalia Hernández-Bellido[2], Laura Ordovás[2,3], Jesús del Barrio[1] and Silvia Hernández-Ainsa[1,3]

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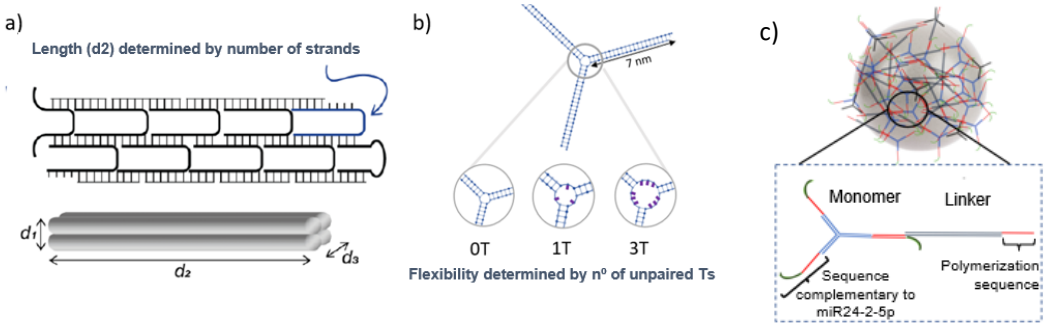
DNA is a biopolymer with excellent self-assembling properties due to its highly specific nitrogen bases recognition. This characteristic enables DNA nanotechnology the use of DNA strands as building blocks to form complex DNA-based nanostructures with total control in their size and shape, in a reproducible way. These DNA nanostructures (DNanos) can be used to encapsulate and protect anticancer drugs and other therapeutic agents and transport them to their action site, increasing their selectivity and effectiveness.[1,2] In this work, we present novel DNanos aimed to trap and deliver anticancer drugs as well as to bear anti-miR sequences for cardiac therapy.

Regarding anticancer drugs, a collection of DNanos with different small and precise variations related to their length (figure 1a) or flexibility (figure 1b) were developed. We aimed to investigate the effect of these variations on their biostability as well as on their ability to internalize into cells, and hence to impact on their drug delivery capabilities. We observed that all the nanostructures were correctly assembled and were capable of encapsulating then anticancer drugs doxorubicin and trifluoperazine. Besides, all of them were resistant to serum nuclease degradation for up to several days and they showed optimal thermal stability (>37ºC) for subsequent cellular studies. Interestingly, we observed these parameters to affect their biostability, their ability to interact with tumor cells and to induce cytotoxicity after drug trapping, consequently affecting their properties as nanocarriers.

Concerning anti-miR cardiac therapy, DNanos were designed to capture miR24-2-5p, a miR involved in heart aging whose reduction leads to therapeutic effect (figure 1c). [3] We have seen correct DNanos assembly, miR24-2-5p capture, as well as internalization and lack of toxicity in HEK293 cells and cardiomyocytes.

Overall, our data suggest the importance of controlling length or/and flexibility in DNanos to tune their biostability and cellular recognition, in order to increase anticancer drugs effect. Besides, our anti-miR designed DNanos present good capabilities for cardiac aging treatment. Currently we are studying their suitability to trap the targeted miR inside the cells and to evaluate their therapeutic effect.



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Synthesis and characterization of hybrid nanomaterials for enhanced metallo prod-drugs delivery.

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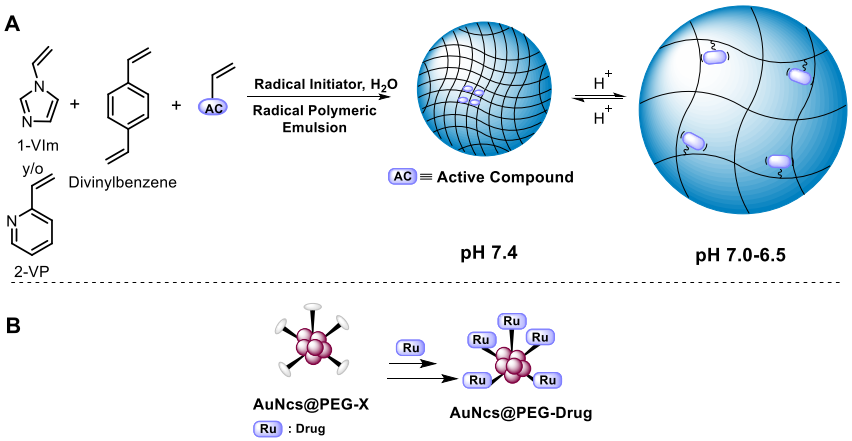
The application of nanotechnology in medicine is giving promising results towards new drugs and personalized therapy, thus achieving increased selectivity, drug delivery efficiency, lower doses, or less side effects. Most recently, Hybrid nanomaterials (NMs) are standing out due to their major technological potential and multifunctional properties compared to conventional NMs, based on a single type of material.
Therefore, in this work it is reported the construction and characterization of hybrid NMs based on metallopro-drugs and pH reversible Polymeric Nanogels (NGs) or gold Nanoclusters (AuNcs).

The pH reversible Polymeric NGs was prepared by Radical Polymeric Emulsion of 1-vinyl imidazole (1-Vim) or 2-vinyl pyridine (2-VP) derivatives, an Active Compound (AC) with a therapeutic effect, which also contains a vinyl group, and divinyl-benzene as cross-linker. (Figure 1A) The NG will be designed to possess an expansion-contraction pH characteristic of carcinogenic extracellular environment. It will be loaded with a drug using the procedure developed by our group [1], followed by drug load and release studies.

On the other hand, AuNCs with novel aromatic dithiol ligands have also been prepared and combined with ruthenium complexes. These dithiol ligands were attached to a poliethylenglicol chain which function is the stabilization of the NM in physiological conditions (Figure 1B). The photoluminescence and quantum yield of the nanomaterial will be measured, as well as the maximum pro-drug load that the NMs accept without losing their luminescence properties.



[1] Nano Lett. 2011, 11, 3136-3141.

Silicon oxide microparticles with covalent and non-covalent immobilization of porphyrin present enhanced singlet oxygen generation and relevant cell uptake

Thais Fedatto Abelha[1], Javier Ruiz-Navarro[3], Consuelo González-Manchón[3], Gordon Bruce[1], David Limón[1], Sandra Giraldo[1], Mariano Redondo-Horcajo[3], Marta Duch[4], José A. Plaza[4], Teresa Suaréz[3] and Lluïsa Pérez-García[1,2]*

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Microparticles (MPs) present useful biomedical applications, ranging from barcodes that enable embryonic identification[1]to scaffolds for photosensitizers commonly used for photodynamic therapy (PDT).[2] We have previously reported that porphyrin immobilized on polysilicon MPs showed enhanced singlet oxygen ($^1O_2$) generation than the free photosensitizer.[2] Motivated by these promising results, we investigated the functionalization of silicon oxide MPs with a covalent and non-covalent immobilization of porphyrin. Both types of functionalization led to bright MPs (Figure 1), which was sustained after washing with acetone and water. The release of porphyrin from MP wafers kept in relevant biological media was negligent for covalently linked porphyrin, while a 20% decrease in fluorescence was observed for the non-covalent labelling. Porphyrin immobilized on silicon oxide MP surfaces generated enhanced singlet oxygen generation than the free photosensitizer. MPs with both functionalizations were internalized by human cervical carcinoma cell line (HeLa). Overall, we report the feasibility of silicon oxide microparticles functionalization both with covalently linked porphyrin and non-covalent label of the photosensitizer, which outperformed the singlet oxygen generation capability of the free photosensitizer, whilst enabling cellular uptake in relevant human cell culture model.

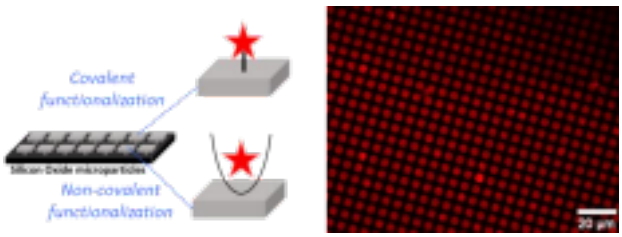

Figure 1: MP functionalization with porphyrin leads to bright particle surfaces (left).

Acknowledgement: This work was supported with funds from the Spanish Government Grant PID2020-115663GB-C32 funded by MCIN/AEI/10.13039/501100011033. TFA also thanks the Universitat de Barcelona, the Spanish Ministry of Universities, the NextGenerationEU plan, the European Union and the Spanish national Recovery, Transformation and Resilience plan.

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Accessible synthesis of silica DNA "nanofossils": applicability in gene storage/preservation and delivery

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Silica nanoparticles have been used as efficient vectors for drug delivery due to their accessible tunability and high biocompatibility.[1] The use of silica nanomaterials as gene delivery vectors has been already explored based on electrostatic conjugation of negatively charged DNA with positively charged surfaces in modified silica nanoparticles.[2-3]

Herein, a direct encapsulation method of pDNA in silica nanospheres is described by using a modified Stöber procedure. This direct method allows the obtention of monodispersed spheres of 350 nm (TEM) with plasmid DNA inside. The encapsulation was confirmed by particle dissolution/DNA isolation and fluorometric assays. These pDNA@SiO_2 particles were tested *in vitro* showing efficient cell uptake, intracellular dissolution and gene release and translation which ended in positive cell transfection. Additionally, encapsulated pDNA functionality was preserved even after treating pDNA@SiO_2 nanoparticles with biological and physicochemical DNA denaturizing agents (ROS, high temperature and light) due to the strong protection that SiO_2 offers.

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Acknowledgments

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Lipid nanocarriers for prevention of biofilm formation in cystic fibrosis patients

Eva Arroyo-Urea[1], María Lázaro-Díez[2], Junkal Garmendia[2,3,4], Fernando Herranz[1,3,4], Ana González-Paredes[1,4*]

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Pseudomonas aeruginosa is a biofilm forming gram negative pathogen associated with progressive and ultimately fatal chronic respiratory infection in cystic fibrosis patients. Biofilms are collective structures of bacteria adhered to a surface which are characterized for being inherently resistant to antibiotics. The conventional treatment of biofilms represents a challenge, and the search for new therapies has become a priority. Recently, the use of nanoparticles as drug carriers has received considerable attention due to their excellent properties, such as high biocompatibility, long stability, and controlled drug release. In this work, we propose an innovative therapeutic approach based on the design, synthesis, and characterization of lipid nanoparticles (LNs) as carriers of a key molecule to prevent P. *aeruginosa* biofilm formation. These LNs, named nanoemulsions (NEs), were constituted by an oily core further stabilized with Tween 80 surfactant (Figure 1a). We designed a set of experiments that employed different lipid to surfactant ratios using the solvent injection technique. Physicochemical properties of these LNs were determined by dynamic light scattering. Most of the synthesized NEs presented small hydrodynamic sizes, homogeneous populations, and positive zeta potential. The platform with the best physicochemical properties was selected as optimal and stability studies were carried out at 4ºC, showing excellent results. Both size and morphology of these NEs were also studied by transmission electron microscopy (TEM) (Figure 1b). To study their potential to prevent P. *aeruginosa* biofilm formation, NEs were tested in ATCC 10145 strain. *In vitro* studies revealed that NEs significantly enhanced the inhibitory effect on biofilm formation of the encapsulated molecule compared to the free drug, demonstrating the effectiveness of using nanoparticles as drug carriers. The blank nanoplatform, used as a control, showed a slight antibiofilm effect, which may be due to the presence of Tween 80 in its composition. These promising *in vitro* data, alongside with their versatility for the loading of different drugs and their excellent stability profile, make these NEs very promising platforms for antibiofilm therapy.

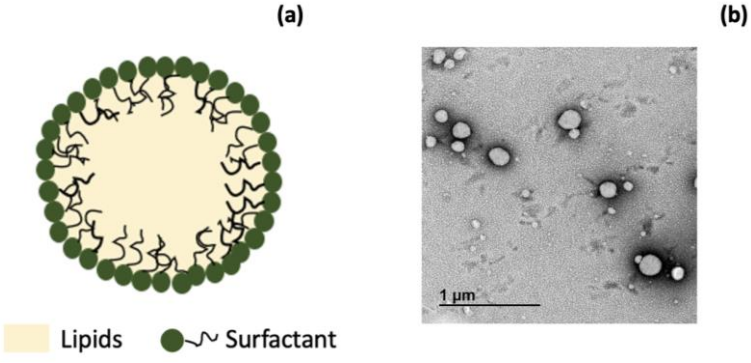

Figure 1. (**a**) Nanoemulsion. (**b**) TEM image of optimized nanoemulsions.

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Microfluidic synthesis of drug nanocarriers for crossing cellular and non-cellular biological barriers

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Lipid nanoparticles are among the most versatile and biocompatible nanocarriers in encapsulating a broad variety of therapeutic molecules of different natures such as mRNAs or doxorubicin for clinical applications.[1] The recent success of SARS-CoV-2 vaccines are partially due to the late advances in rapid-mixing methods such as microfluidic mixers for large scale production of lipid nanoparticles.[2] This work aims at the development of synthetic protocols with a microfluidic system and the ethanol-injection method, to design lipid nanoparticles with different physicochemical properties (e.g. size, surface charge and composition) to study the biophysicochemical interactions at the nanobio-interface. In particular, we are interested in targeting the brain and the lung. The main problem for treating neurological diseases lies in the difficulty of crossing the blood-brain barrier (BBB). Although its function is necessary for protection against toxic substances and pathogens, it is a double-edged sword since it does not allow the transfer of drugs.[3] Contrary, the lungs are mainly protected by non-cellular barriers such as the mucus or the lung surfactant. We aim at producing a library of lipid nanoparticles labeled with contrast agents for monitoring by MRI and PET the crossing of biological barriers and the interaction with the lung surfactant and the mucus for targeted delivery of therapeutics.

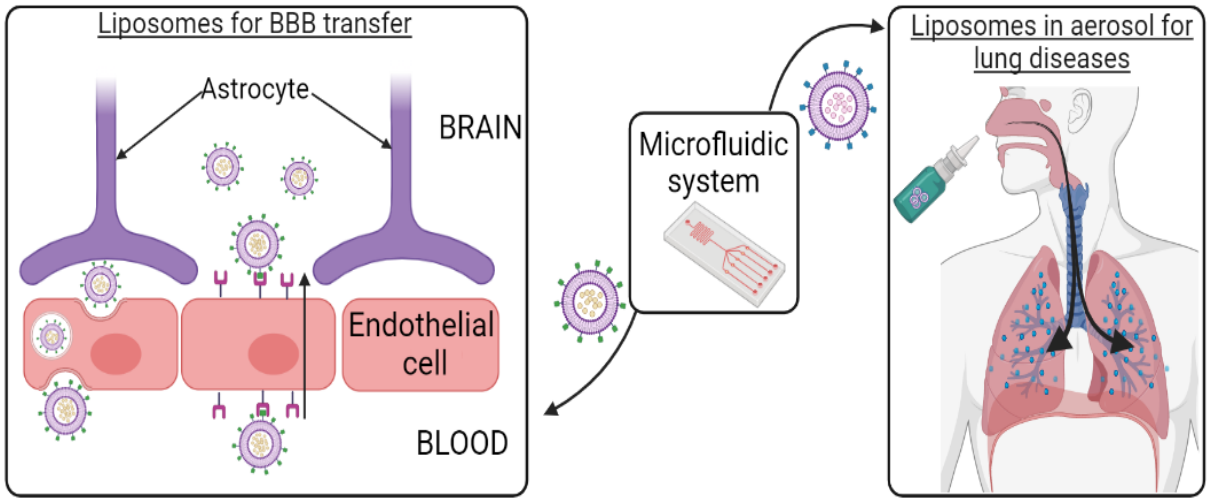

Figure 1: Schematic diagram of the manufacturing method and the two main applications

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Bioorthogonal click chemistry on living cell membranes: creating hotspots for transient increase of living cell membrane permeability

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Heat generation by magnetic nanoparticles (MNPs) under exposure to an alternating magnetic field (AMF), known as magnetic hyperthermia (MH), has traditionally been studied for cancer treatment applications, focused on the internalization of MNPs in target cells. Herein, we developed an innovative approach to apply localized heating onto living cell membranes for inducing changes of membrane biophysics. Our approach is based on the covalent immobilization of MNPs on the cell membranes via bioorthogonal click chemistry, more specifically the strain promoted [3+2] azide-alkyne cycloaddition (SPAAC) between azide-labelled cell membranes and strained alkyne-functionalized MNPs.

First, the expression of azide reporters on human colorectal carcinoma cells (HCT116) was optimized through metabolic glycoengineering. Then, 13 nm iron oxide MNPs were functionalized with two different types of passivation molecules: polyethyleneglycol (PEG, 750 Da) and a glucopyranoside derivative (Glc) to increase colloidal stability and biocompatibility. In a second functionalization step, MNPs were decorated with two types of strained alkyne with different reactivity towards azides, namely cyclooctyne (CO) and dibenzocyclooctyne (DBCO). The clickable properties of both types of functionalized MNPs have been characterized in suspension via the reaction with 3-azido-7-hydroxycoumarin. Furthermore, the reaction with azide-coated gold substrates has been quantified with quartz crystal microbalance in water and in cell culture conditions. Finally, studies of the interaction of these MNPs with azide-modified cells have been conducted via fluorescence microscopy and flow cytometry, showing a higher cell membrane retention time of the PEG coated MNPs.

Upon the application of an AMF, these MNPs acted as "hotspots" to generate a very localized heating of the cell membrane, leading to changes in cell membrane fluidity that promoted the intracellular delivery of a membrane-impermeable fluorescent probe, the YOPRO-1, without compromising cell viability. We therefore anticipate that our approach could be adapted to a wide variety of therapeutic applications, in particular cell transfection.

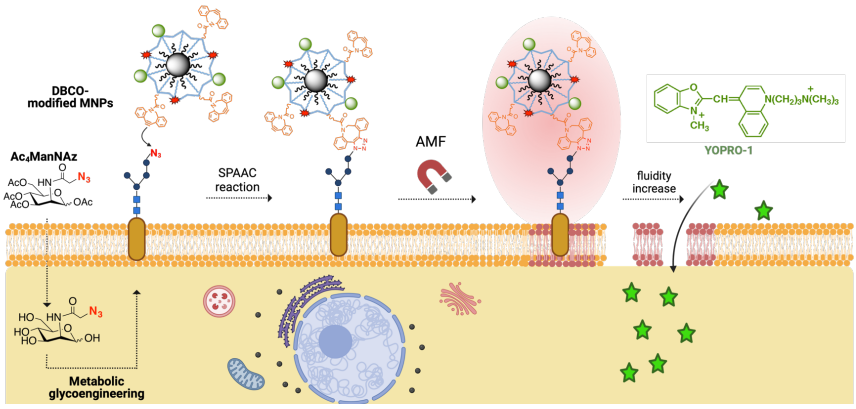

Figure 1. *Scheme of the application of localized MH onto cell membranes for YOPRO-1 intracellular delivery.*

Hyperbranched Polyglycerol Prodrug of Methotrexate is an Effective Nanomedicine for the Treatment of Rheumatoid Arthritis in a Preclinical Model

Urs O. Häfeli*, Zeynab Nosrati, Yun An Chen, Marta Bergamo, Cristina Rodriguez-Rodriguez, Katayoun Saatchi

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Inflammatory arthritis is a group of diseases characterized by inflammation of the joints and often other tissues including rheumatoid arthritis. Rheumatoid arthritis (RA) patients are frequently prescribed anti-arthritic drugs including nonsteroidal anti-inflammatory drugs, glucocorticoids, disease-modifying anti-rheumatic drugs and biologics, or combinations thereof. Many patients fail to respond satisfactorily to these treatments because only a small percentage of the dose reaches the target. In addition, many patients experience serious side effects. Improving the uptake of the existing drugs in the joints might reduce the dosing frequency and increase therapeutic effects with less toxicity.

Herein we report a theranostic nanomedicine prodrug with long circulation time and sustained release of the drug in the inflamed joints. Pharmacokinetics studies using radiolabelling techniques and SPECT/CT imaging showed that the theranostic nanomedicine approach delivers higher concentrations of anti-arthritic drugs to inflamed joints than has previously been possible, despite lower and less frequent drug doses (**Figure 1**). The efficacy of the prodrugs was established in the same mouse RA model and compared to the free drug given in the same form and timing as is currently administered to patients. In vitro stability measurements of the prodrugs in human synovial fluid from rheumatoid arthritis patients help to better understand the impact of the local environment of an inflamed joint and how that influences drug release.

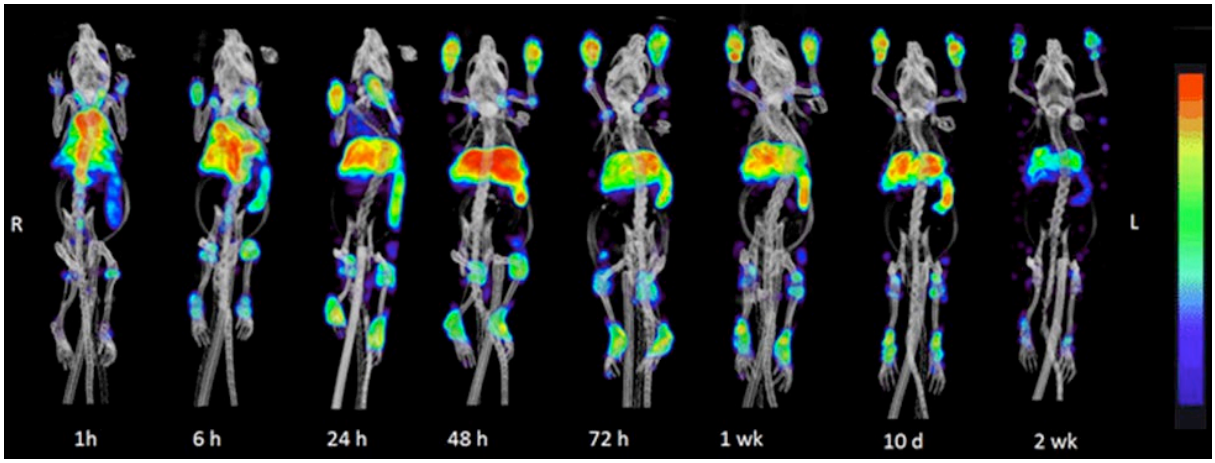

***Figure 1**. Radiolabelled biodistribution of HPG-methotrexate in an RA mouse model over two weeks.*

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Exploring iron oxide nanoparticles for neural regeneration

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In recent years, nanomaterials including magnetic nanoparticles are emerging as attractive tools for the development of new and more effective therapies to treat neural disorders, specially aiming to the regeneration of the central nervous system. However, despite numerous advances, a better understanding of the properties of these materials and how they influence their ability to therapeutically interact with cells and tissues to attain their maximum benefit is still required. In this work, we have examined the response of primary neural cells to novel designs of flower-like superparamagnetic iron oxide nanoparticles obtained by "bottom-up" approaches in an open dissolution process (NFD) or in an autoclave (NFA). By using immunofluorescence techniques, intracellular calcium dynamics and lipidomics, the formation of functional neural networks has been analyzed. In addition, by transmission electron microscopy and flow cytometry, it has been verified that neural cells are capable of internalizing both types of nanoparticles and remain viable, being these phenomena dependent on the concentration used, as well as on the incubation time and type of nanoparticle selected. The influence of applying an alternating magnetic field on these neural cell cultures in the presence of NFA and NFD has been also explored. These studies have confirmed the preservation of highly interconnected neural networks under these stimulation conditions. Importantly, synaptophysin and β-III tubulin increase in cultures exposed to the combined treatment, thus indicating the existence of more mature synapses.

These findings demonstrate the biocompatible interaction of these magnetic nanoparticles with primary neural cells and their potential to be considered as attractive candidates for driving regeneration in neural tissues. Furthermore, they lay the groundwork for unravelling the molecular mechanisms driving such interactions.

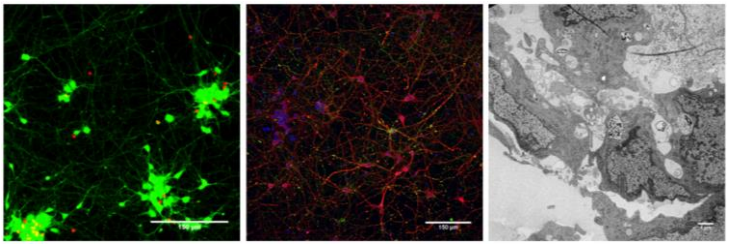

Figure 1. Representative images of viability (left; green for live cells and red for dead cells), differentiation (middle) by confocal microscopy (neurons in red, positive for β-III tubulin, synapses in green, positive for synaptophysin, and cell nuclei in blue, labeled with Hoechst) and cell uptake by transmission electron microscopy (right) of neural cell cultures exposed to NFA nanoparticles (0.01 mg Fe/mL). Scale bars: 150 µm (left and middle) and 1 µm (right).

Acknowledgments

This work was supported by the *Ministerio de Ciencia e Innovación* and the *Fondo Europeo de Desarrollo Regional* (PID2020-113480RB-I00) and CSIC (PIE 202160E060).

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Approaching neural regeneration by using magnetic responsive hydrogels made of natural polymers

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Annually, spinal cord injuries affect 250000-500000 people worldwide. Despite this significant incidence, an effective treatment for these patients does not exist yet due to the complexity and limited regeneration potential of the central nervous system, among other factors. For decades, clinicians and researchers have been working on developing new regenerative approaches for these pathologies.
Here, we evaluate the use of magnetic hydrogels derived from natural polymers and magnetic iron oxide nanoparticles, that combine suitable properties for biomedical application,[1,2,3] as novel platforms to drive neural repair in the injured spinal cord.

First, magnetic nanoparticles were coated with natural polymers such as chitosan (IONP-CHI) by ionic gelation with sodium tripolyphosphate (non-toxic polyanion). Natural hydrogels composed of collagen were prepared with IONP-CHI to become magnetic by a freeze-casting methodology. IONP-CHI presented high colloidal stability, while collagen hydrogels were mechanically stable, highly porous and fibrillary. Preliminary cell assays with primary neural cells showed tight interactions between neural cells, IONP-CHI and collagen hydrogels. Indeed, IONP-CHI promoted high cell viability and maintained neural cells capacity to differentiate into neurons up to 0,05 mg Fe/mL. Furthermore, they allowed differentiated neurons to form intricated cultures similar to control conditions. Magnetic collagen hydrogels also supported high cell viability and allowed neuronal differentiation up to 0.1 mg Fe/mL of IONP-CHI. Glial cell differentiation was modulated by the presence of IONP-CHI and collagen.

To sum up, magnetic collagen hydrogels and chitosan-coated nanoparticles prompt positive biological responses in primary neural cells. Some of their properties need to be optimized to fully unravel the actual regenerative potential of these biomaterials.

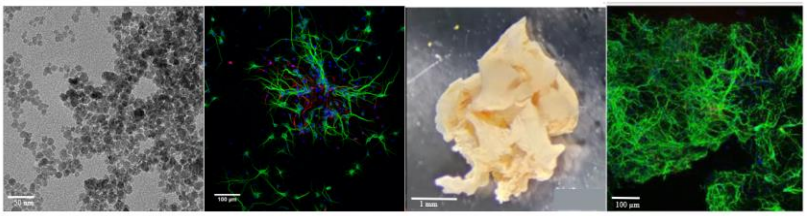

Figure 1. a,c) IONP-CHI and collagen hydrogel by electron and optical microscopies, respectively; **b,d)** Representative confocal images of primary neural cells exposed to (b) 0.05 mg Fe/mL of IONP-CHI and (c) magnetic collagen hydrogels. Neurons appear in green labelled for MAP-2, glial cells in red labelled for vimentin and cell nuclei in blue by Hoechst staining.

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Core@shell nanostructured electrodes for neural interfacing

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Nowadays, aiming to provide more efficient therapeutic alternatives, neuroscience, nanotechnology and materials science disciplines are working together on the development of novel neural interfaces of improved properties and efficiency.

In this work we have explored the possibility of combining the properties of different materials in the same structure by designing and fabricating metallic flexible nanostructured electrodes with core@shell nanowires, being only the shell exposed to the environment. We have established a novel approximation, following a multi - step procedure, to obtain electrodes with vertical Ni@Au nanowires in which the initial flexible electrode, covered with a network of vertical nanowires made of Ni (a robust material), is obtained by template-assisted electrodeposition and posteriorly covered by a biocompatible Au shell obtained by pulsed electrodeposition, with fine control on the shell thickness.

Morphological and structural characterization showed the Au shell uniformly covering the Ni core, confirmed as well in cyclic voltammetry studies, since no remarkable Ni electrochemical activity was detected. In addition, these nanostructured electrodes present reduced impedance when compared with flat ones. Finally, *in-vitro* biocompatibility studies highlight the capability of the Ni@Au electrodes to mediate biocompatible responses *in vitro*.

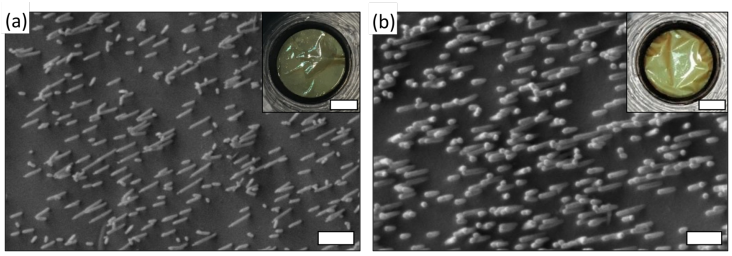

***Fig. 1**. SEM top view of (a) bare Ni - NWs electrode, (b) after the Au shell growth (Ni@Au – NWs electrode). Scale bars in SEM images: 1 µm, and in insets: 0.5 cm.*

FUNDING SOURCES: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 737116. Acknowledgment to UCM Predoctoral contract (CT17/17-CT18/17).

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Synthesis and characterization of semiconductor nanoparticles as luminescence nanothermometers in mice eyes

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One of the first signs of many diseases and health dysfunctions is the appearance of local temperature variations, such as fever or hypothermia. [1] At present, recording temperature changes at the nanoscale in deep areas constitute an early diagnostic method. For this purpose, luminescent NPs can be used as nanothermometers in luminescence nanothermometry (LNT), a non-invasive technique that provides information about temperature variations at the nanoscale. [2] The aim of this work is to study the diagnostic power of NPs for ocular diseases using LNT.

In this project, silver sulfide (Ag_2S) nanoparticles (NPs) are used as luminescent nanothermometers capable of obtaining, without contact, temperature readings through the change in their photoluminescence emission intensity when embedded in biological tissues, specifically in mouse eyes. These synthesized Ag_2S NPs are biocompatibilized by a surface modification procedure prior to intravitreal injection into explanted eyes. Figure 1a shows the change of luminescent signal intensity of the NPs (1 μL) inside mouse eyeballs with increasing temperature. Figure 1b shows optical and NIR images of the eyes. In addition, in this project we have performed cell culture studies (Figure 1c) to evaluate the impact of the NPs at the retina.

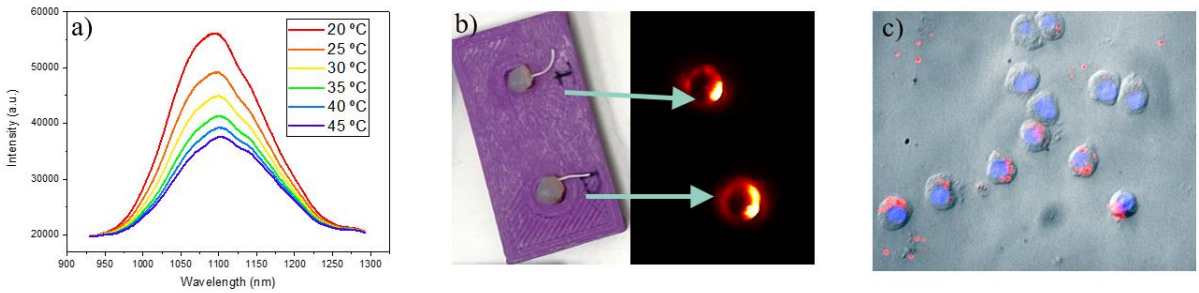

Figure 1: a) Change in intensity photoluminescence emission with temperature of biocompatible NPs inside a mice eyeball; b) injected eyeballs and their image taken with an infrared camera; c)microscopic image of cells found in mice retina with NPs.

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In-depth Study of Rare-earth Nanostructures to Design Efficient Luminescent Nanothermometers for Biological Applications

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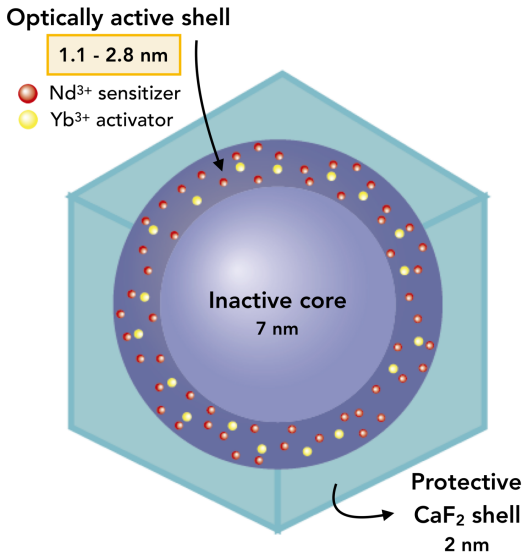
Non-invasive techniques for *in vivo* temperature readout have a wide interest because deviations from the basal body temperature are important indicators of pathological (*e.g.* infection-induced inflammation) and physiological (*e.g.* metabolic activity) processes [1]. Rare-earth luminescence nanothermometers, more concretely $NaYF_4@NaYF_4:Nd_{60\%},Yb_{20\%}@CaF_2$ nanoparticles, are ideal candidates for a precise and reliable temperature evaluation *in vivo* because their excitation and emission match the biological windows, within the Near-Infrared region [2].



In order to design more efficient nanothermometers, it is necessary to understand in depth how the arrangement of the dopants (Nd^{3+} and Yb^{3+}) in the matrix structure influences their spectroscopic properties. For this reason, we have carried out a systematic study of these nanocrystals by changing the thickness of the intermediate optically active shell, which is confined between an inactive core and an external protective shell. The emission intensity, luminescent decay time curves, quantum yield and thermal sensitivity are measured for all core/shell and core/shell/shell nanocrystals. The results reveal the great influence that the nanoparticle architecture imposes over the spectroscopic performance of the nanocrystals. In fact, nanocrystals with a 2 nm-thick active shell and protective outer shell exhibit the longest lifetime and the highest quantum yield (~15%) and thermal sensitivity (~1.1%). This information is supported by a theoretical model that nicely reproduces the thermal sensitivity response of these nanocrystals, which can be used for the design of new and more efficient thermal probes.

Acknowledgements: We acknowledge the Spanish MINECO MAT2017-83111R, Comunidad de Madrid B2017/BMD-3867 and REACT PR38/21 ANTICIPA-UCM for the support. I.Z.G. thanks UCM-Santander for a predoctoral contract (CT63/19-CT64/19).

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Quantitative detection of SARS-CoV-2 RNA using a compact fluidic electrochemical biosensor platform

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This work reports on the development of a compact analytical platform for the quantitative detection of SARS-CoV-2 RNA specific sequences in nasopharyngeal swabs without the need of RNA purification and amplification. It comprises a biosensor approach based on the use of a miniaturized two-electrode electrochemical cell and a paper fluidic component, that also incorporates magnetic nanoparticles (MNPs) functionalized with Polypurine Reverse Hoogsteen hairpins that selectively and sensitively interact with the target sequences of the virus. The functionalized MNPs were firstly incubated with the sample in the presence of a reporter DNA sequence conjugated to a peroxidase enzyme (HRP), outside the biosensor platform. In this step, the RNA specific sequences were captured by the MNPs, also reacting with the reporter DNA sequence. Then, the MNPs were added to the analytical platform for carrying out the electrochemical detection by a very simple chronoamperometric approach, which relied on the HRP reaction with H_2O_2 in a solution also containing ferrocenemethanol redox mediator. The overall analysis time was around 40 min. Cathodic currents were recorded that increased with the target RNA sequence concentration in buffered solutions. The lowest concentration that unambiguously produced a signal different from that of the blank solution was 0.01 nM (1 fmol). The platform was applied in a retrospective study to the analysis of 58 nasopharyngeal swab samples provided by the IGTP. Considering the RT-PCR results as true positives and negatives, the electrochemical biosensor showed a sensitivity and specificity of 86 %. The biosensor performance just required a compact and battery-powered instrumentation, connected to a smart phone. All these features make the presented biosensor analytical tool to be of potential implementation for decentralized analysis at the point-of-care.

This research work was funded by the European Commission – NextGenerationEU (Regulation EU 2020/2094), through CSIC's Global Health Platform (PTI Salud Global)

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Radio frequency impedance monitoring of inductive sensors for quantification of COVID-19 immunoresponse.

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The COVID-19 pandemic has shown the importance of rapid diagnosis and its accessibility to preserve life and safety. Lateral flow immunoassays (LFIA) also known as rapid tests, have been used to detect the infection. As the pandemic evolved and population got vaccinated, new bio-sensing needs arise. At this point the challenge is to monitor the immune response to optimize the vaccination process and effectiveness.

The immunoresponse of vaccinated or infected people requires quantification of immunoglobulin M (IgM) and immunoglobulin G (IgG) to monitor the immunoresponse to the COVID-19. Visual inspection of traditional LFIA does not allow precise quantification given the low penetration of light. Inductive radio-frequency measurements allow precise and high sensitivity quantification. The best nanotag for bio-sensing volumetric samples are magnetic nanoparticles MNPs. These magnetic lateral flow immunoassays (MLFIA) have been used satisfactorily for multiple applications [1][2]. Previous MLFIA can quantify only one type of bio-molecule. We developed a multi-MLFIA that can, in one test, measure IgM and IgG concentration.

The MNPs present on the rapid test modify the effective permeability of the inductive sensor. Under specific conditions this increase in effective permeability is traduced on an impedance increase on the inductive sensor. The measurements of the multi-MLFIA is done on an inductive sensor with a specialized shape. The increased area of the inductive sensor used on the multi-MLFIA setup decreases the sensitivity slightly. Even with a decrease in sensitivity the multi-MLFIA setup duplicates the speed and the reliability of the measurement procedure.

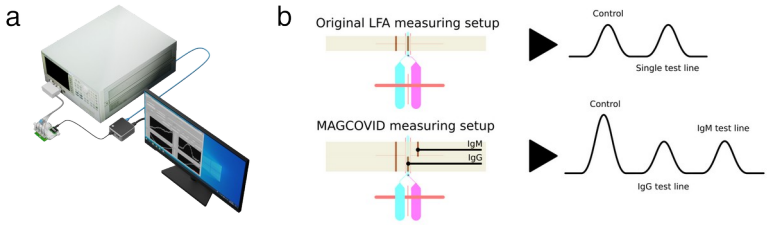

Figure 1. a) Inductive sensing setup consisting in impedance analyzer and micro-positioning system. b) Sensor setup and new multi-test measuring sensor.

Acknowledgments: This work was partially founded by the University Technological Institute of Asturias (IUTA) under grant SV-21-GIJON-03, and the Government of the Principality of Asturias under projects FICYT/IDI/2021/000100 and FICYT/IDI/2021/000273.

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Iron oxide and iron oxyhydroxide nanoparticles exert anti-viral activity in SARS-CoV-2-infected Vero E6 cells

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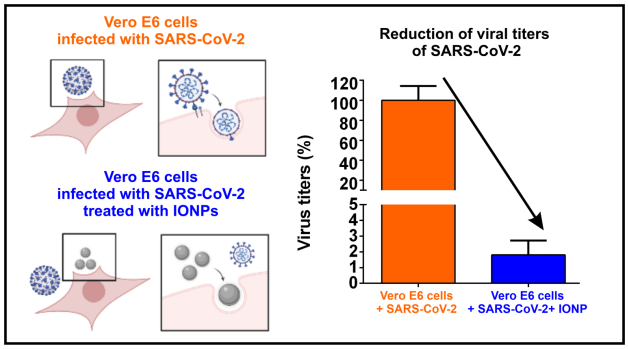
Coronaviruses usually cause mild respiratory disease in humans. Nevertheless, some human coronaviruses are able to cause more severe diseases, such as the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has spread globally since December 2019, resulting in the ongoing coronavirus pandemic.

In this study we have analyzed the potential use of iron oxide nanoparticles (IONPs) coated with biocompatible molecules such as dimercaptosuccinic acid (DMSA), 3-aminopropyl triethoxysilane (APS) and carboxydextran (FeraSpin™ R), as well as iron oxyhydroxide nanoparticles (IOHNPs) coated with sucrose (Venofer®), and iron salts (ferric ammonium citrate (FAC)), in the treatment and/or prevention of SARS-CoV-2 infections. Used at non-cytotoxic doses, IONPs and IOHNPs impaired SARS-CoV-2 replication, transcription and production of infectious viruses in vitro, either when Vero E6 cells were treated previous or after the infection, although with different efficiencies. Our data suggest that SARS-CoV-2 infection affect the expression of genes involved in cellular iron metabolism. Furthermore, the treatment of cells with IONPs and IOHNPs affect the oxidative stress and iron metabolism to different extents, likely affecting virus replication and production.

Interestingly, some of the nanoparticles used in this work have already been approved for their use in humans as antianemic treatments, such as the IOHNP Venofer®; and in small animals, such as mice, as contrast agents for magnetic resonance imaging (MRI), such as the IONP FeraSpin™ R. Therefore, our results suggest that the IONPs and IOHNPs, may be repurposed and used as prophylactic and therapeutic treatments against SARS-CoV-2 infections.



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Nanoparticles as a tool for management of biofilm-associated infections

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Biofilms are communities of bacteria attached to surfaces and embedded in an extracellular matrix that the bacteria themselves synthesize, which is a mechanism of antibiotic resistance since it increases the resistance of bacteria and favors the horizontal transfer of resistance genes, giving rise to chronic infections that are difficult to treat [1]. As antimicrobial resistance (AMR) is one of the biggest global threats to public health today, there is an urgent needed of therapeutic alternatives [2], and in this regard, nanoparticles (NP) can offer several advantages [3].

Both metallic nanoparticles and nanostructured lipid carriers (NLC) were investigated for their potential activity against biofilm formation in a gram negative pathogen, *Haemophilus influenzae*. Iron oxide nanoparticles (IONP) and zinc-doped IONP were obtained through hydrothermal synthesis using iron and zinc chloride as metal sources and sodium citrate as surfactant, whereas NLC, which core is a mixture of liquid and solid lipids stabilized with surfactants, were synthetized by the solvent injection technique. Moreover, metallic NP surface was functionalized with a peptide described for its ability to inhibit biofilm formation in other gram negative pathogens. The ability of metallic and lipid NP to inhibit biofilm formation in *H. influenzae* was tested using biofilm crystal violet staining assay.

All nanoparticles obtained resulted in appropriate physico-chemical properties, as shown in table 1, and they were very stable, maintaining their initial physicochemical properties at least for 3 months under storage at different temperatures. Surface functionalization with antibiofilm peptide was achieved only in IONP, without modifying significantly its physicochemical properties. Nevertheless, *in vitro* tests of biofilm formation inhibition showed that peptide-functionalized IONP, zinc-doped IONP and NLC were able to impair biofilm formation in *H. influenzae*, resulting in total inhibition at the highest concentrations tested.

In view of these promising preliminary results, further work for a deep study of their efficacy and biocompatibility will be carried out soon, as the NP here presented are a potential source of alternative treatments for biofilm-associated infections

Table 1. Initial physico-chemical characteristics of nanoparticles studied

Nanoparticles ID	Hydrodynamic size (nm)	PDI	Z potential (mV)
IONP	6,5 ± 0,2	-	-17,2 ± 6,6
Zn-doped IONP	5,2 ± 0,3	-	-36,8 ± 3,1
NLC	171± 4	0,157± 0,012	-20,0 ± 4,1

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Modulating the layer-by-layer nanoarchitectonics via the incorporation of nanoencapsulated enzymes to perform enzymatic cascade reactions with potential biomedical applications

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Enzymatic (bio)chemical cascade reactions play a pivotal role in the regular cell functioning. The alteration of these cascade reactions may have a detrimental effect on cell homeostasis, growth and differentiation. Hence, the recovery of those aberrant cellular pathways becomes a fundamental factor in the restoration of the (bio)chemical stability. In the present work, the combination of the layer-by-layer (LbL) approach with individually nanoencapsulated enzymes (SENs: single enzyme nanogels) is envisioned as a potential strategy to formulate novel microreactors capable of performing enzymatic cascade reactions with potential therapeutic applications (Fig. 1A). As a proof of concept, glucose oxidase (GOx) was encapsulated in the cavity of LbL polymeric microcapsules, which were subsequently decorated on their surface with nanoencapsulated catalase (SENs-CAT) (Fig. 1B). As determined by confocal microscopy, SENs-CAT were successfully incorporated during the LbL process, being preferentially accumulated on the surface of the capsules (Fig. 1C). The resulting capsules were able to independently scavenge H_2O_2 and reduce glucose levels from solution in an efficient manner, confirming that the enzymes remained active after the fabrication process (Fig. 1D). Finally, the capacity of these microreactors to perform enzymatic cascade reactions was proven by measuring the levels of H_2O_2 produced by the first enzymatic reaction (i.e., Glucose – GOx → H_2O_2 + gluconic acid), which was subsequently scavenged in the second enzymatic reaction (i.e., H_2O_2 – CAT → O_2 + H_2O). In view of these preliminary results, the combinatorial approach presented herein could be considered in the future for the treatment and replacement of damaged cellular pathways. The authors of the present work acknowledge the funding from the Basque Government (PIBA_2021_1_0048) and *Fundación Eugenio Rodriguez Pascual*.

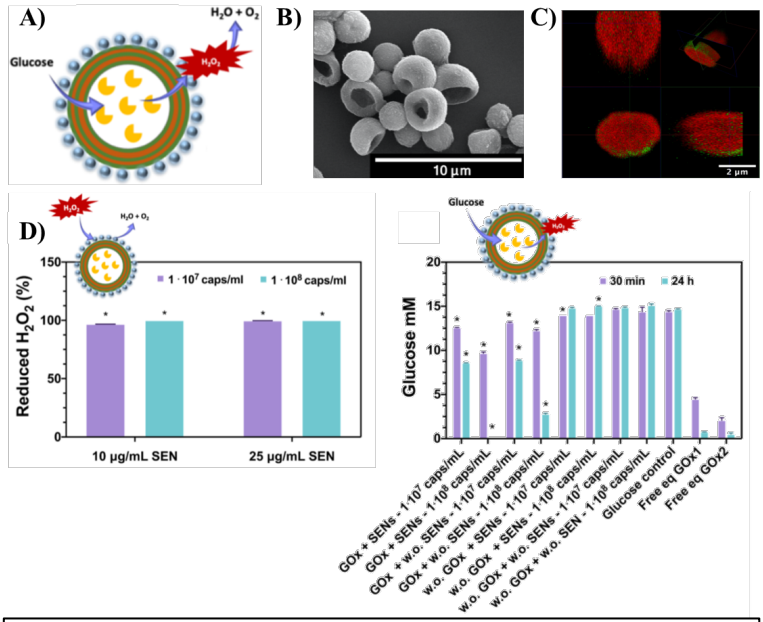

Fig. 1. *A) Schematic representation of the LbL capsules containing GOx in their cavity and SENs-CAT on their surface. B) SEM micrograph of the resulting capsules. C) Confocal micrographs of the capsules loaded with GOx (red) and decorated with SENs-CAT (green). D) Capacity of the capsules to scavenge H_2O_2 and glucose from the solution.*

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Hybrid-tag polymeric nanotrackers as universal reagents for long-term live-cell barcoding

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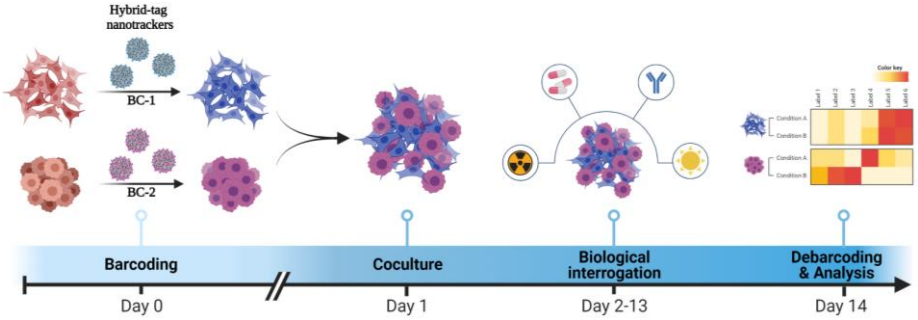
Barcoding and pooling cells for processing as a composite sample is critical to minimize technical variability for multiplex technologies. Fluorescent cell barcoding has been established as standard method for multiplexing in flow cytometry analysis. In parallel, mass-tag barcoding is routinely used to label cells for mass cytometry, a cutting edge multi-parametric single-cell platform that allows the detection of nearly 60 markers per single cell. Mass cytometry comprises conventional flow cytometry and inductively coupled plasma time-of-flight mass spectrometry (ICP-MS). Mass cytometry uses metal-tagged reagents, such as metal chelating polymers instead of fluorescent-labelled reagents. Nanomaterials such as polystyrene nanoparticles, lanthanide-coordinated semiconducting polymer dots (Pdots), and inorganic nanoparticles are getting an increasing attention for mass cytometry. Barcoding of samples allows controlling variability between samples and fluctuations in machine sensitivity. Barcode reagents in current use label intracellular proteins in fixed, permeabilized cells and therefore are not suitable for studies with live cells in long-term culture prior to analysis. In this study, we report the development of fluorescent palladium-based hybrid-tag nanotrackers to barcode live cells for flow and mass cytometry dual-modal readout. We describe the preparation, physicochemical characterization, efficiency of cell internalization and durability of these nanotrackers in live cells cultured over time. In addition, we demonstrate their compatibility with standardized cytometry reagents and protocols. Finally, we validated these nanotrackers for drug response assays during a long-term coculture experiment with two barcoded cell lines. This method represents a new and widely applicable advance for fluorescent and mass-tag barcoding that is independent of protein expression levels and can be used to label cells before long term drug studies.



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A Magnetic Nanoparticle-Based Vaccine Generates Anti-Tumour Immunity *in vitro* and *in vivo*

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Cancer is one of the biggest medical problems worldwide. In fact, it is the second leading cause of death around the globe. Immunotherapy, which consists of directing the patient's immune system against this disorder, has become a major focus for its treatment[1]. One possible strategy consists in the development of cancer vaccines that deliver tumour antigens and adjuvants to antigen-presenting cells. The latter may then induce a potent and sustained T-cell response and promote the effective infiltration of those cells in the tumour microenvironment[2].

Herein, we developed a cancer vaccine based on magnetic nanoparticles (MNP) functionalized with a model peptide antigen (a cysteine-modified class I restricted epitope of ovalbumin, Cys-OVA$_{257-264}$, COVA) and an adjuvant (CpG oligonucleotide). The resulting formulation (MNP-CpG-COVA) was fully characterized (e.g., TEM; **Figure 1a**) and its biocompatibility with blood and bone-marrow dendritic cells (BMDC) was assessed *ex vivo*. *In vitro*, MNP-CpG-COVA activated BMDC, evidenced by high expression of maturation cell surface markers CD86, CD40 and MHCII), and efficiently trigger the presentation of the antigen to CD8+ T-cells. As a result, CD8+ T-cells significantly proliferated and were activated to kill the melanoma cells expressing ovalbumin (B16-OVA) in an antigen-specific manner (**Figure 1b**). The immune response triggered by MNP-CpG-COVA was also assessed *in vivo*, and demonstrated remarkable results in terms of CD8+ T-cells activation (e.g., higher proportion of effector T-cells, **Figure 1c**) and therapeutic efficacy against the tumour.

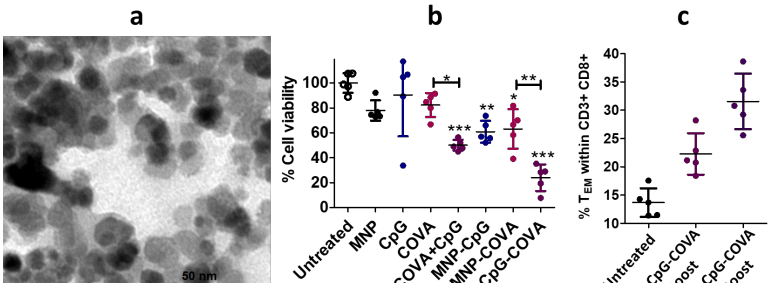

Figure 1.- a) TEM image of MNP-CpG-COVA. b) Cell viability of B16-OVA cells after 72 h of coculture with BMDC and CD8+ T-cells. c) Percentage of T effector-memory cells in mice's blood 21 days after the first injection of MNP-CpG-COVA.

In summary, we thoroughly explored the potential use of modified MNP as a cancer vaccine platform and demonstrated its efficacy *in vitro* and *in vivo*.

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Targeting Tumor Endothelial Marker 8 in metastatic disease

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Current anti-angiogenesis therapies for inhibiting the progression of metastatic disease are mainly based in targeting endothelial markers such as Vascular Endothelial Growth Factor receptor (VEGFR). However, these therapies have shown limited benefits in clinics as they result in undesirable side effects because they interrupt both physiological and pathological angiogenesis. In the searching for new tumoral vasculature markers, the Tumor Endothelial Marker 8 (TEM8), an integrin-like cell surface receptor, has gained attraction in recent years. It is enriched in tumor endothelium *vs* non-malignant endothelium, and in some cancer cells in a wide variety of solid tumors. This makes it a useful tool for specifically targeting tumor endothelial cells in the growing tumoral organ.
In this work, we use nano-biotechnology in order to specifically direct nanomaterials carrying therapeutic drugs to tumoral cells overexpressing TEM8. For that purpose, we have engineered a TEM8 natural ligand, the Anthrax Toxin, to design a ligand-protein (10xHis:PA17) for targeting this receptor. We used this ligand-protein for stable electrostatic biofunctionalization of Doxorrubicin (DOX) loaded mesoporous silica nanoparticles (DOX@MSNs@10xHis:PA17). We show that this straightforward biofunctionalization is stable under physiological conditions and that at the same conditions, the drug is sustainedly released over time, unlike current therapies. We have also developed a metastatic melanoma murine model by intraperitoneal transplant of B16F10 cells to direct our DOX@MSNs@10xHis:PA17 to the TEM8 vascular receptors. We hope this targeting and therapeutic strategy will be a promising alternative to overcome common limitations of conventional treatments for metastatic disease.

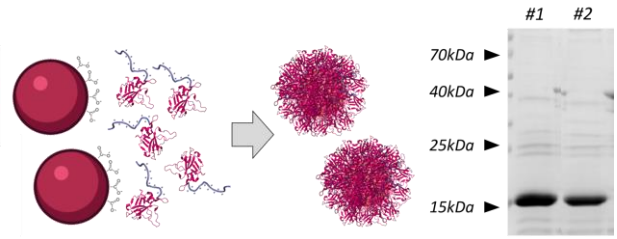

Figure 1. Scheme of MSNs electrostatic biofunctionalization. Coomassie blue stained-gel of ***#1*** MSNs@10xHis:PA17 ***#2*** DOX@MSNs@10xHis:PA17. In the stained gel, our protein of interest is the most abundant one bound to MSNs surface by electrostatic interactions.

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Acknowledgments

-Instituto de Salud Carlos III (ISCIII) Grant PI19/00349 co-funded by the European Regional Development Fund, "Investing in your future".

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Visual and Multiplexed Detection of MicroRNAs Using Functionalized Gold Nanoparticles

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Cancer is the second leading cause of death worldwide, being responsible for 1 in 6 deaths globally. In this sense, early diagnosis of cancer is crucial for the success of treatments and the reduction of cancer-associated mortality. For that, different biomarkers have been used in oncology, with varying levels of accuracy and efficacy.

Among those, microRNAs (miRNAs) are promising candidates, as they can be detected in liquid biopsies and their dysregulation is associated with early stages of cancer progression. However, currently established miRNA detection methods, are complex, costly, require specialized personnel and sophisticated equipment, limiting their application in point-of-care settings or resource-limited facilities. Recently, nanotechnology-based approaches, in particular using gold nanoparticles (AuNPs), have emerged as promising alternatives.

In this work, the unique optical properties of AuNPs are explored for the development of colorimetric sensors for miRNA detection. Such systems should allow simple, fast and low-cost detection, being suitable for handling by patients or non-specialized professionals. We propose the use of 10-13 nm AuNPs functionalized with probe oligonucleotides bearing a cholesterol moiety. In our system, a thiol group is placed at one end of the oligonucleotide, to ease the conjugation with AuNPs, and a cholesterol derivative is placed at the other, to achieve target-driven modulation of the colloidal stability of the nanostructures. Thus, the presence of the target miRNA can be detected by a decrease in the color intensity of the solution. We apply this system in the diagnosis of very aggressive cancers, such as uveal melanoma, pancreatic cancer and breast cancer.

Our system has shown good sensitivity and selectivity *in vitro*, allowing the detection of target sequences with the naked eye in few hours. Furthermore, this sensor can recognize specifically several miRNAs at a time, with picomolar sensitivities. It shows excellent performance in biological samples, like the human serum, being suitable for direct determination in liquid biopsies and potential use in early cancer detection in the point-of-care (PoC).

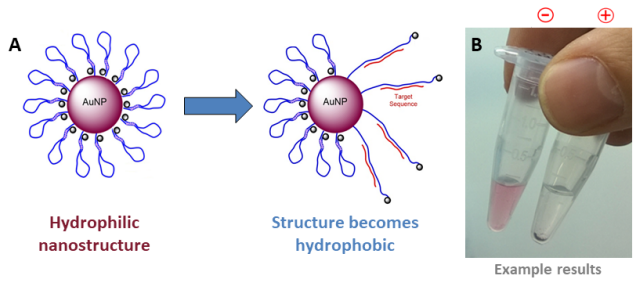

Figure 1 – A) Schematic representation of the miRNA sensor, composed of AuNPs functionalized with oligonucleotides. B) Left – sample with nanoparticles dispersed in water; right – aggregated nanoparticles in the presence of the target sequence.

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MAPSULES: Multimodal theranostic agents for externally controlled and non-invasively monitored nanotherapies.

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Nanoparticles have not fulfilled yet the expectation in cancer treatments due to poor accumulation and clearance. New multifunctional nanomaterials enabling external control of the accumulation and non-invasive visualization and detection of the therapeutic action could give new prospects to the field. Here we present novel magnetoplasmonic drug-loaded (paclitaxel) biodegradable nanocapsules based on metallic iron semishells (MAPSULES) merging highly efficient external actuation with magnetic fields and near infrared light to locally boost the therapeutic action[1,2].

The MAPSULES are engineered by a combination of bottom-up and top-down techniques. Briefly, monodisperse drug loaded poly-lactic-co-glycolic-acid (PLGA) cores of 150 nm are partially coated by Fe/X (X=SiO_2, Au, Ti) by combining colloidal self-assembly and physical vapour deposition. The outer layer can be tuned to exploit different effects such as X-ray imaging or enhanced photothermal efficiency.

The MAPSULES exhibit ferromagnetic vortex configuration (i.e., zero net magnetic moment at zero field), enabling high colloidal stability and strong magnetic manipulation. The magnetophoretic force are *ca.* 300-fold larger than in superparamagnetic iron oxide nanoparticles. The nanocapsules exhibit very intense r_2 relaxivity (370 $mM^{-1}s^{-1}$) in MRI, i.e., much higher than commercial iron oxide contrast agents. The metal Fe semishell exhibits highly damped plasmonic behavior with intense broadband absorbance in the near infrared (NIR), leading to an excellent photothermal conversion efficiencies in the 1st and 2nd biological windows.

Remarkably, MAPSULES *in vivo* therapeutic assays in a mouse xenograft tumor model show a high amplification of the therapeutic effects by combining magnetic concentration and photothermal actuation in the tumor. These results highlight the strength of this externally controlled and amplified therapeutic approach, which could be applied to locally boost a wide variety of drugs for different diseases.

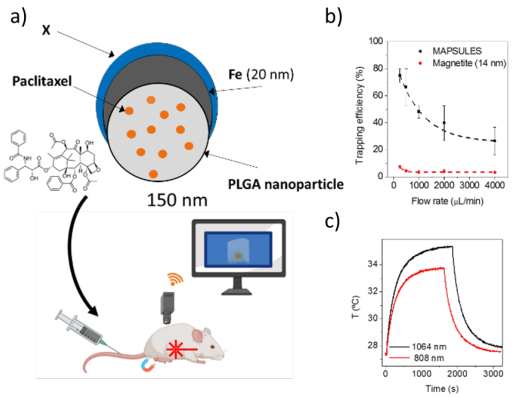

Figure a) Schematic of the drug loaded ferromagnetic nanocapsules components and their functionalities. b) Magnetic trapping efficiency in a microfluidic channel at increasing flow rates c) Demonstration of the efficient optical heating in the 1st and 2nd biological windows.

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Multifunctionalization strategies on nanoparticles for diagnostic chemical proteomics

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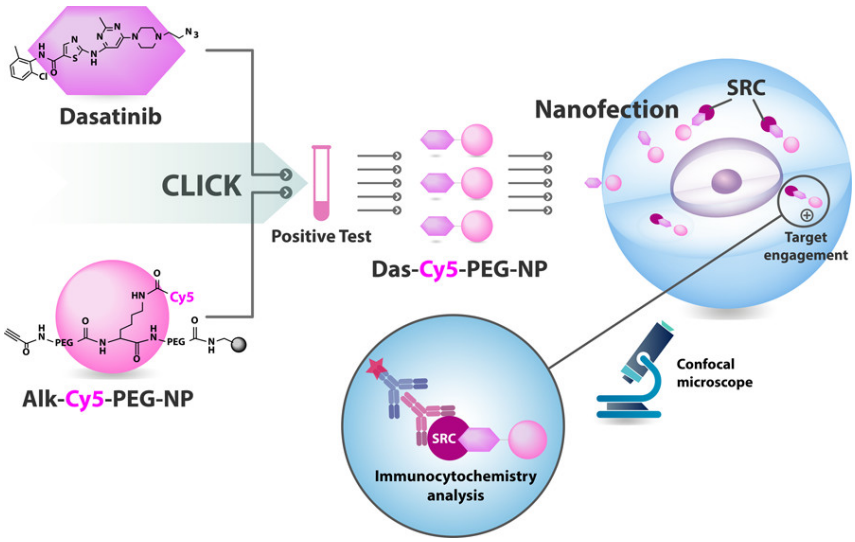
Cancer is the second highest cause of death in Europe, with 3.7 million estimated new cases and 1.9 million deaths each year. One of the main strategies in recent cancer therapy has been the development of promiscuous multi-target drugs, aimed to circumvent resistance mechanisms. Target promiscuity also represents a major drawback due to the appearance of severe side effects, which limit the dosage and efficiency of the treatment. Finding the spectrum of targets of each anti-cancer drug in each specific cell type or patient is the key for precision cancer therapy, since most effective drugs with reduced side effects can be individually selected. This contribution tackles this issue through the development of a multiplexing method based on nanotechnology to detect target proteins for a given drug. We present a straightforward procedure for the conjugation of azide-tagged drugs to miniaturized solid supports by click chemistry, along with an analytical method to rapidly determine whether the coupling reaction has been successful. As a proof of concept of the strategy, the synthesis, characterization, and biological validation—*in vitro* and *in cellulo*—of a novel cell-penetrating nanodevice decorated with the tyrosine kinase inhibitor (TKI) dasatinib is reported.



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Increasement of Oxidative Stress by IONPs alters mitochondrial bioenergetics and mitochondrial dynamics in cancer cells

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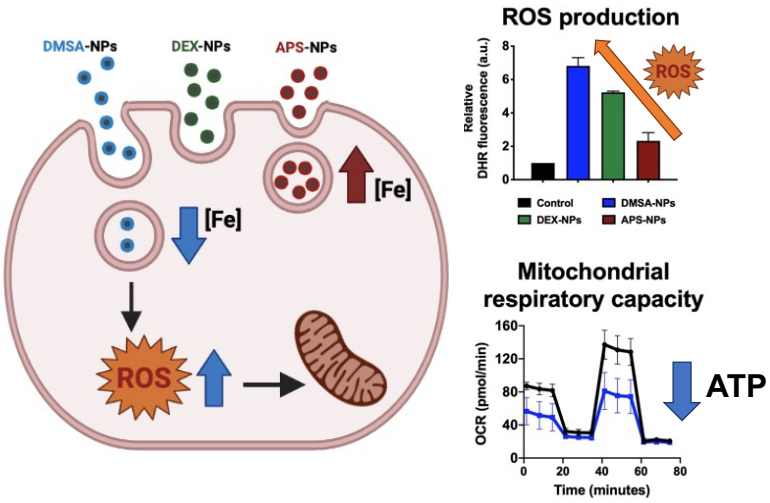

Cancer cells are characterized by having a dysregulation on the redox state, thus, inducing changes in the intracellular amount of reactive oxygen species (ROS) is becoming a potential target for cancer therapy. Due to their properties, iron oxide nanoparticles (IONPs) can produce ROS inside cells through Fenton Reaction, therefore, there is a growing interest on using these nanoparticles to affect the redox status of cancer cells.

Therefore, we explored the different effect of DMSA-coated, DEX-coated and APS-coated IONPs to induce oxidative stress in two tumoral cell lines, an adenocarcinoma cell line (Pan02) and a breast cancer cell line (MDA-MB-231). After incubation with IONPs, ROS production was studied over time and the contribution of oxidative stress on affecting mitochondria functionality and distribution was assessed.

Although APS-NPs were highly internalized in cancer cells, DMSA-NPs were the ones which had the highest capacity to produce ROS inside cells. This increasement of oxidative stress, lead to changes in mitochondrial functionality and disposition. DMSA-NPs were able to hamper the function of mitochondrial respiratory chain, thus, reducing the capacity to produce ATP through the Electron Transport Chain (ETC). Moreover, besides changes in cell metabolism, DMSA-NPs were also able to induce changes in mitochondrial dynamics, as mitochondria showed a more elongated disposition after incubation with DMSA-NPs in both cell lines, but only induced mitophagy in the breast cancer cell line.

Mitochondrial dynamics is actively involved in both tumorigenesis and metabolic plasticity that allows cancer cells to adapt to unfavorable environment. Our results showed that the induction of oxidative stress using DMSA-NPs, could be used as an innovative approach to not only affect cellular metabolism, but also reprogram mitochondrial dynamics in cancer cells.

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Inner-particle magnetization dynamics in relation to magnetic chaining/unchaining

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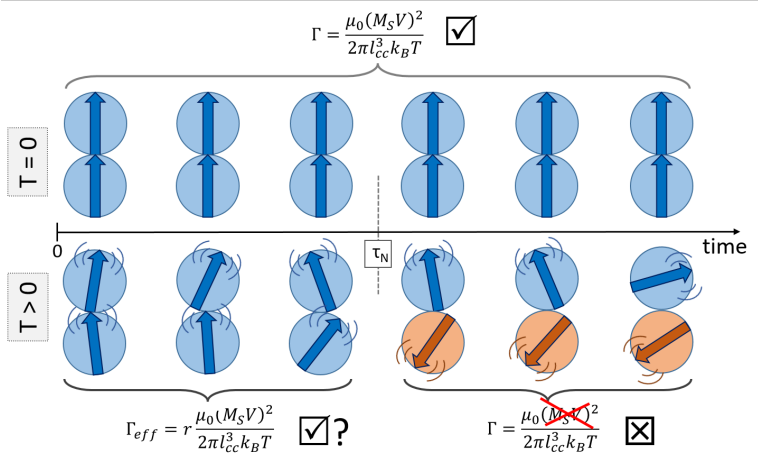
Magnetic colloids have huge potential for a variety of applications, as the nanoparticles may be used as small *nanorobots* for delivering various stimuli, as heat (hyperthermia treatment, catalysis) or mechanical actuation (magnetogenetics). Triggering the desired mechanism requires accurate control of the particle response, which is mainly defined by two parameters: the particle magnetic moment (which couples it to the field); and its anisotropy (which couples the moment to the lattice). The second one is particularly relevant, as it is responsible for converting the field stimulus into the required action, e.g., heat or torque. At the same time, for the particles to produce effective performance, their behavior as a colloidal system must be also well controlled. And in that sense, it is critical to control its agglomeration likelihood, either if desired (agglomeration may enhance the desired triggering stimulus), or if undesired (it may also cause blood clots). The problem is that in the scientific literature, the magnetization dynamics -governed the anisotropy- and the colloidal properties are often treated separately: either the magnetization dynamics is applied over a frozen colloid, or the colloidal properties are ascribed to the rigid dipole approach (infinite anisotropy limit). In the current work we will show, by combining a simple analytical interpretation in terms of characteristic relaxation times and one-dimensional Monte Carlo simulations, why both aspects need to be simultaneously considered. By starting from the colloidal properties approach, we will focus on the problem of estimating the likelihood of stable agglomerates to form, or to break apart; for simplicity we will focus on chain-shape agglomerates.



Sketch illustrating the rigid-dipole limit where the usual estimate for agglomeration likelihood, Γ, works (equivalent to T=0 K); and the more realistic situation in which thermal fluctuations weaken the interparticle coupling strength, and may eventually break it.

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Magnetic Nanoparticle transformations and the effect on their heating properties

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In the Magnetic Hyperthermia (MH) and Photothermal Therapy (PTT) applications, magnetic nanoparticles (MNPs) are used as an "antennae" able to capture energy (either from an alternating magnetic field (AMF) or a near infrared (NIR) light) and transform it into local heat. In the frame of this therapy design, it is fundamental to know how possible particle transformation would affect its performance over time.

In this work, we used MNPs with two different coatings (dimercaptosuccinic acid - DMSA-NPs) and poly(maleic anhydride-alt-1-octadecene) - PMAO-NPs) but with same magnetic core (≈13.5 nm) (Figure 1A). We evaluated how the MNP coating affects their degradation profile using a medium that simulates the lysosomal conditions and how this degradation affects their heating performance in the frame of both magnetic hyperthermia and photothermal treatments. The faster degradation of DMSA-NPs in comparison with PMAO-NPs was verified by transmission electron microscopy (TEM), magnetic and colorimetric measurements (Figure 1B).

To track how the transformations suffered by the particles along their degradation process affected their heating properties, magnetic hyperthermia and photothermal measurements were performed (Figure 1C). In both cases, the degradation process resulted in a decrease of the heating capacity of both types of materials. As a result of the faster degradation of DMSA-NPs, the reduction of the heating properties along time was increased for this material when compared to PMAO-NPs.

Thus, the less prone to degradation nanoparticles (PMAO-NPs) were selected for the *in vivo* analysis, to evaluate the degradation speed of this material in tumor tissues. In this study, although the number of particles decreased in the tumors along time after their administration, no transformations in the average particle size of particles occurred (Figure 1D).

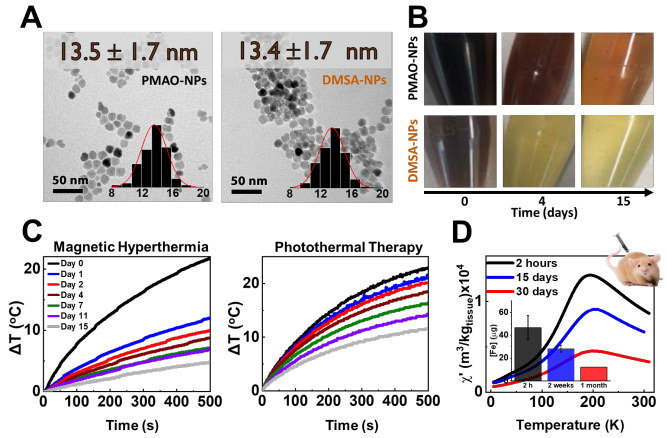

Figure 1. A) TEM images and particle size distribution of the nanoparticles used. B) Photos of the nanoparticle suspensions at different times during the degradation process. C) Magnetic Hyperthermia and Photothermal measurements of PMAO-NPs at different times of the degradation process. D) Temperature dependence of the AC magnetic susceptibility profiles of tumor tissues collected at different time points and iron concentration in the form of particles in the tumor calculated from the out-of-phase susceptibility data.

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Nanorod Photothermal Therapy Targeting Head and Neck Cancer Using a Modified Toxin Natural Ligand

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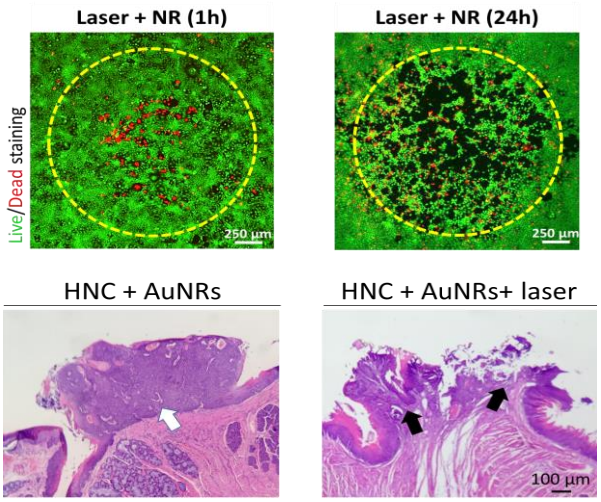
Head and neck squamous cell carcinoma is the sixth leading cancer in the world. Currently, HNC is diagnosed by a physical examination followed by an histological biopsy, with surgery being the primary treatment, often followed by adjuvant radiotherapy or chemoradiotherapy. However, unfortunately, local recurrence of HNC squamous cell carcinoma is common .
We have shown that preneoplastic and malignant HNC cells and tissues from different origins aberrantly exhibit the glycosphingolipid globotriaosylceramide (known as GB3 or CD77) on their membranes[4]. This receptor specifically binds to the Shiga toxin (ShTxB), which is a high-affinity innocuous natural ligand that, upon binding to the receptor, triggers retrograde receptor internalization through the Golgi network to the endoplasmic reticulum.
To mimic natural ligand-specific cell entry mechanisms, we have reproduced the molecular cues found in the Shiga toxin to target nanomaterials into HNC cells bearing the GB3 receptor. We functionalized gold nanordos (AuNRs) with this toxin (AuNRs@ShTxB) and we find that are efficiently retrotranslocated to the cancer cell cytoplasms. After laser radiation with a wavelength resonant with the AuNR longitudinal localized surface plasmon, the death of targeted cancer cells is activated. Both *in vitro* and *in vivo* experiments show the non-cytotoxic nature of these functionalized nanoparticles and a selective treatment after laser treatment. This functionalization strategy, is a clear example of how some toxin fragments can be used as natural biosensors to target nanomedicines to head and neck lesions.



Acknowledgments
Instituto de Salud Carlos III (ISCIII) Grants PI19/00349 and CD19/00035 co-funded by the European Regional Development Fund, "Investing in your future". IDIVAL project: INNVAL20/13.

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Searching candidates for Magnetic Hyperthermia Therapy through shape and size rational design of MNPs

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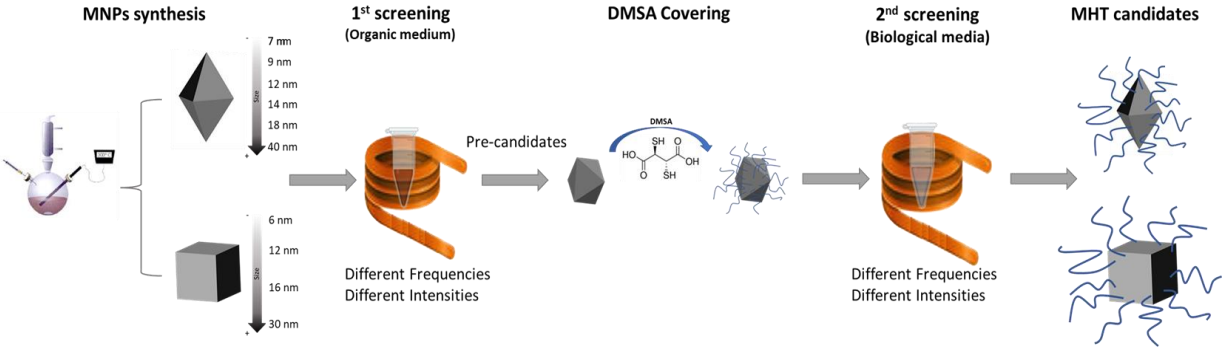
Traditional hyperthermia is commonly used in combination with chemotherapy or surgery during cancer treatment. However, side effects are usually generated due to non-specific application. Consequently, magnetic hyperthermia therapy (MHT) has emerged as a promising solution. MHT is based on the intrinsic capacities of magnetic nanoparticles (MNPs) to accumulate by EPR effect or by specific target molecules in the tumor and to respond to alternating magnetic fields (AMF) by releasing heat. Focusing on this second issue, the ability of MNPs to release heat, through Néel and Brown relaxation, depends on intrinsic and extrinsic parameters. Regarding the intrinsic parameters, a rational design of NPMs is necessary to maximize the capacity to release heat, with size and shape, and consequently anisotropy, being crucial. Additionally, the extrinsic parameters are related to the operating conditions of the equipment, basically, frequency and intensity of the magnetic field.

In this work, we synthesized different shapes and sizes MNPs: 6, 9, 12, 14, 18 and 40 nm octahedra MNPs and 7, 12, 16 and 30 nm cubic MNPs. Then, SAR values were calculated and compared between them using two different equipment, which allow us a wide range of operating conditions. On the one hand, magneTherm™ (from nanoTherics©) give us the possibility to test different frequencies: 178 – 1009 kHz (but the magnetic field intensity is limited to 20 mT). On the other side, the Five Celes© magnetic hyperthermia equipment provides us the possibility of testing different magnetic field intensities: 0 – 60 mT (but frequency is restricted to 282 kHz). After an exhaustive screening, we selected the most promising MNPs to continue the study. Consequently, we transferred these MNP to aqueous medium through meso-2,3-dimercaptosuccinic acid (DMSA) covering and evaluated the maintenance of heat release capacity in different biological media including DMEM with 10% FBS over time and in presence of high cells concentration.



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Spanish Conference
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Development of multifunctional nanoplatforms based on highly stable iron oxide nanoparticles for therapeutic applications.

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Finding novel strategies for effective diagnosis and treatment of diseases is one of the main objectives in the clinical field. The use of nanotechnology has brought new tools for the development of advanced materials. Specifically, nanoparticles can penetrate cell membranes and be remotely manipulated to produce a healing effect, helping to formulate more promising and safer approaches with higher therapeutic efficacy [1]. Magnetic nanoparticles are a major class of nanomaterials due to their high potential in biomedicine, such as efficient agents for hyperthermia treatment, drug delivery and magnetic resonance imaging [2-3]. Hyperthermia therapy is an anticancer clinical practice based on elevation of the tumor temperature, driving malignant cells and tissues up to the cytotoxic level, that is, 43-48 °C. In addition, cell resistance against traditional treatments, such as chemotherapy or radiotherapy, can be temporally reduced [4]. In this work, we report the synthesis of a multifunctional drug delivery system based on magnetic iron oxide nanoparticles (IONPs) combined with a chemotherapeutic agent, doxorubicin (DOX), suitable for multimodal hyperthermia-based anticancer treatments. The stability of these DOX-loaded IONPs in the tumor environment has been characterized by X-ray absorption spectroscopy (XAS) at the Fe K-edge (7112 eV). Through analysis of the spectra at the XANES regime, any changes in the phase or composition of iron in the NPs after chemo-functionalization and subsequent tumor cell internalization can be detected. On the other hand, the EXAFS analysis provides information about their structural order, number of neighboring atoms and distance at which they are found. After a detailed examination, we can conclude that XAS characterization reveal that DOX-loaded IONPs are highly stable when internalized into tumor cells.

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Aknowledgements: This work was supported by the Spanish Ministry of Economy and Competitiveness (SEV-2016-0686, RED2018-102626-T, FPI SEV-2016-0686- 20-1, FPU18/02323), Comunidad de Madrid (2017-T2/IND5395 Talento, 2018-T1 / IND-1005 Talento, 2018 / NMT-4321), AECC (project Ideas Semilla 2019) and H2020 European project (No 685795. NoCanTher). Special thanks to BM23 (ESRF) beamline staff for the support during the experiments.

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Non spherical magnetite nanoparticles for biomedical applications

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Magnetic nanoparticles are a tool with great prospects for the future. They can improve various treatment methods such as hyperthermia, targeted drug delivery and MRI treatments to mentions just a few.

In term of hyperthermia, the magnetic nanoparticles can produce localized heating by when they are subjected to an alternating magnetic field, this is called Magnetic Hyperthermia [1,2]. On the other hand, when the nanoparticles are irradiated by a laser with a certain wavelength, they also obtain heat release in a specific area [3]. Both stimuli can be used simultaneously or separately.

In general, spherical nanoparticles are used for this type of application. However, in this work we wanted to explore the possibilities of elongated EMNPs (ellipsoids) with Au nanoparticles attached to their surface [Figure 1]. For this purpose, 550 nm long nanorods have been synthesized, coated with three layers of polymer to make them biocompatible and gold seeds to improve their optical properties [4,5].

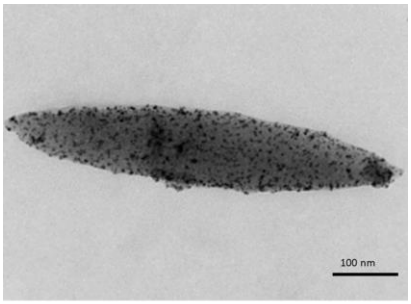

Fig. 1. EMNPs synthesized for this work

In this work, magnetic hyperthermia and photothermia techniques, and a mixture of both, were applied to explore and optimize the parameters of field amplitude, frequency, and irradiation power to obtain a less harmful therapy.

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Reproducible magnetic iron oxide nanocarriers by microwave assisted synthesis

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The most ambitious applications of magnetic iron oxide nanoparticles are in medicine where they can be used for diagnosis, drug delivery, magnetic resonance imaging and cancer treatment [1]. Among the different synthesis methods developed for the preparation of this kind of particles, microwave (MW) technology seems a feasible alternative providing an efficient heating in environmentally friendly media. Traditional heating sources often produce temperature gradients which reduce the effectiveness within energy transfer through the solution. On the contrary, MW irradiation provides a uniform rise in temperature over the whole reaction volume by coupling MW energy to the molecules inside the reaction mixture [2].

Here, we have analyzed the reproducibility of the synthesis of 15 nm iron oxide nanoparticles by microwave assisted synthesis in polyol. Nanoparticles' synthesis was carried out using diethylene glycol as solvent, surfactant and reductant in a microwave and in presence of water to reduce the reaction temperature down to 170 ºC and it was repeated eight times. As shown in Figure 1, the synthesis exhibited great reproducibility (>90 %) for particle size (13-15 nm, ±3), crystal size (15-16 nm), hydrodynamic size (160-190 nm, PDI=0.3), saturation magnetization values over 80 emu/g and the obtained particles are water stable for a long time. Furthermore, the as-prepared nanoparticles were evaluated as drug delivery agents considering ibuprofen as model compound. The maximum ibuprofen uptake as well as its release under an alternating magnetic field was followed by UV/Vis showing promising results.

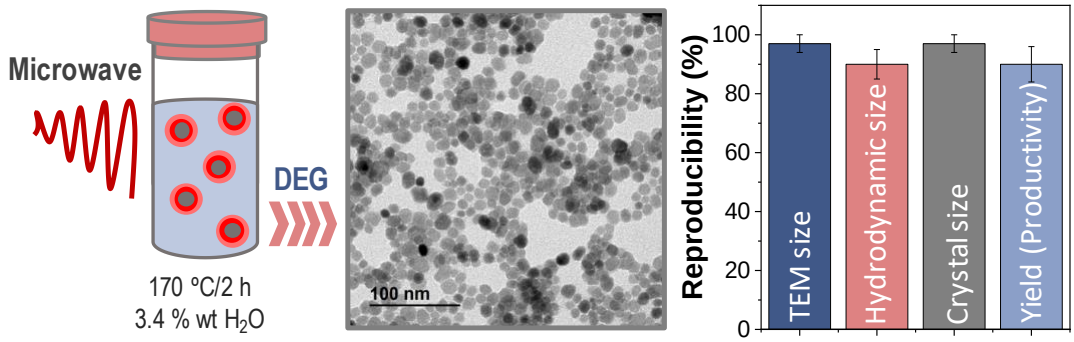

Figure 1. Reproducibility of microwave-assisted synthesis of iron oxide nanocarriers.

Acknowledgment

This work has been partially supported by NESTOR-101007629 EU project (H2020-MSCA-RISE-2020), and IMAGINE project from the European Science Foundation.

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Functionalitation of Iron Oxide NPs Using New PEG-Derived Ligads and Micelles Encapsulation.

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Iron oxide nanoparticles (IONPs) have shown great applications as diagnostic and therapeutic agents in the medical field. The IONPs present extraordinary characteristics as magnetic resonance imaging contrast agents and also the IONPs that present ferromagnetic behavior are able to produce heat by applying alternating magnetic fields (named magnetic hyperthermia).

The synthesis of these type of IONPs are prepared by a very well-known thermal decomposition method, however, the main backdraw of this method is the synthesis of hydrophobic particles[1]. For this reason, it is necessary to develope robust and versatile methods to render water-soluble and stable NPs.

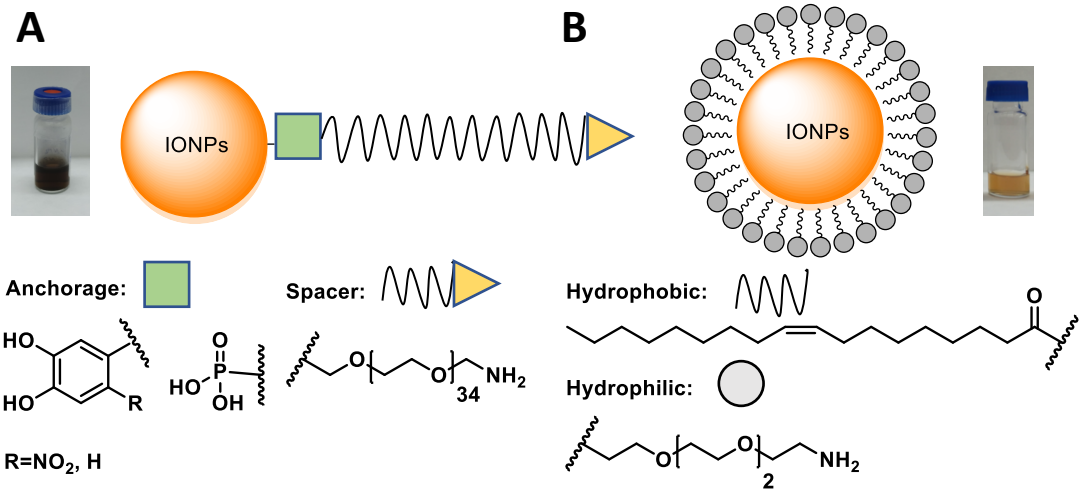
In this report, it has been carried out two different methodologies to functionalize the hydrophobic IONPs:

The ligand exchange method. This protocol was performed with different PEGylated ligands varying the anchorage group, which can be either a catechol or a phosphonic acid derivate.

The encapsulation of NPs. This method was performed to disperse IONPs in aqueous medium by using amphiphiles that present a lipophilic chain based on oleic acid, and a polar head based on TEG. The apolar chains of the amphiphiles interact with the hydrophobic surfactants of the IONPs displaying the hydrophilic head to the water medium.[2]

These functionalized IONPs shown excellent features as MRI contrast agents and also as a potential therapeutic agent against cancer due to the magnetic hyperthermia and the possible surface functionalization for drug delivery.

Moreover, IONPs show excellent properties like chemical stability, low-cost production, surface functionalization, and the possibility to vector the therapeutic molecule to the desired target making them a valuable alternative to the more traditional materials.



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NANO3DEVICES: theranostic nanodevices with translational application.

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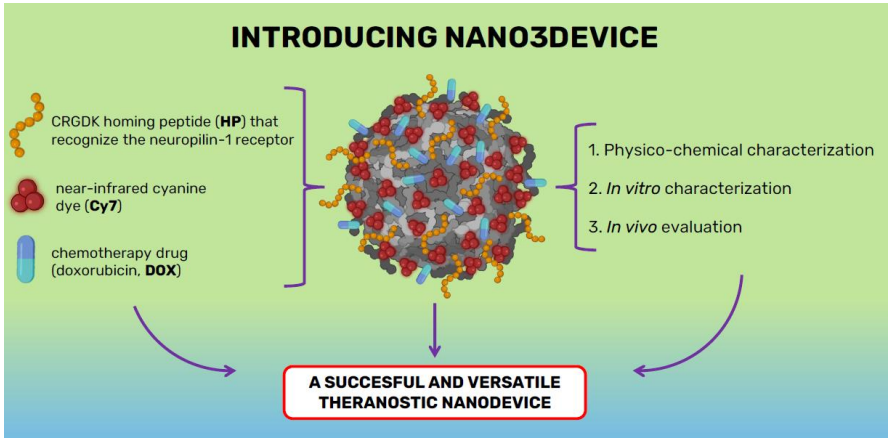
Theranostic nanodevices are capable of both delivering therapy and tracking disease through imaging. We have already validated a novel tri-functionalized nanodevice for cancer theranostics, named Nano3Device. It consists on synthetic nanoparticles carrying a chemotherapy drug (doxorubicin), near-infrared cyanine dye (Cy7) and CRGDK homing peptide, which can actively recognize the neuropilin-1 receptor overexpressed in triple negative breast cancer cells. Now the main objective of this work is to advance towards the validation of an effective, safe, versatile and non-toxic nanodevice for an in vivo use with theranostic application, i.e., for selective and monitored anti-tumor treatment. For that purpose, Nano3Device has been physico-chemically characterized, together with a preclinical evaluation. Such evaluation was conducted both in vitro and in vivo, addressing pharmacokinetics (including tissue distribution), toxicology and pharmacodynamics. The in vivo theranostic potential of Nano3Device has also been investigated, being compared with gold standard first line chemotherapy and more selective treatments such as liposomal-based drug delivery. Preliminary results were obtained from triple-negative breast cancer xenografts (MDA-MB-231 cells in NSG mice), showing that the use of Nano3Device nanoparticles reduces the required dose of chemotherapy, promotes a local action in the area around the tumor and significantly reduces tumor volume without evidence of toxicity. In addition, Nano3Device levels in the tumor, liver and plasma were undetectable after the end of treatment, suggesting that it could be successfully cleared. This novel nanodevice has yet to be validated in different aspects. Once its versatility has been confirmed, a broad range of drugs, trackers and selective peptides or antibodies can be bound, expanding the potential of the nanodevice for cancer treatment. This work has a clear translational focus, facilitating the transition from basic research to the development of clinical applications that lead to better cancer diagnosis and treatment.



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Raman spectroscopic assessment of Hsp90 amyloid refolding capacity

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Neurodegenerative diseases are a major health problem for the world's aging population. Although these debilitating diseases have great differences in their clinical manifestation and prevalence; they also have a common event, the progressive accumulation of misfolded protein aggregates in well-ordered β-sheet structures[1]. These structures are known as amyloid, and are the hallmark of e.g. Alzheimer's, Parkinson's, Huntington's disease or amyotrophic lateral sclerosis (ALS). Amyloid fibrils are filamentous structures approximately 10 nm wide and 0.1 to 10 µm long with a cross-β structure. Currently, the diagnosis of the disease is based on clinical symptoms, generally through a combination of psychiatric questionnaires and biomedical imaging methods that are slow, subjective, and may not be predictive of disease onset[3]. For these reasons we propose Raman spectroscopic techniques to obtain a rapid and predictive diagnosis of the appearance of the disease. It is known that the amide-I band is spectroscopically distinct for an unstructured polypeptide compared to a β-sheet-rich fibrillar aggregate, making it a great biomarker[4]. In this work we evaluated the Heat Shock Protein 90 (Hsp90) capacity to correctly fold unstructured polypeptides by comparing the amide-I band of controls and polypeptides incubated with Hsp90 purified protein.

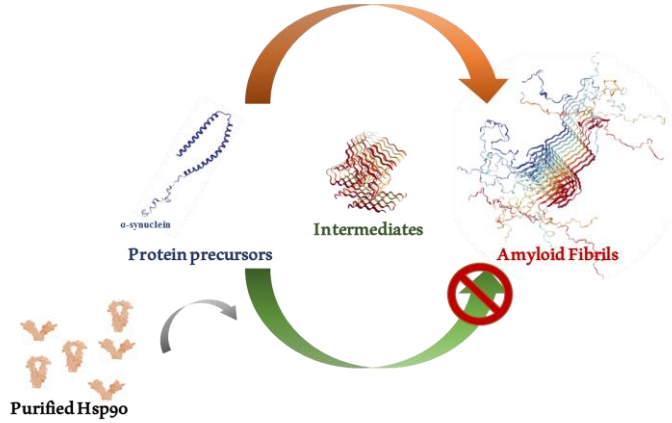

Figure 1. Schematic representation of the expected result after protein precursors incubation with Hsp90.

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Acknowledgments: Instituto de Salud Carlos III (ISCIII) Grant PI19/00349 co-funded by the European Regional Development Fund, "Investing in your future". PREVAL 19/04.

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Neural Stem Cells Differentiation on Densely Packed High Aspect Ratio Nanopillars

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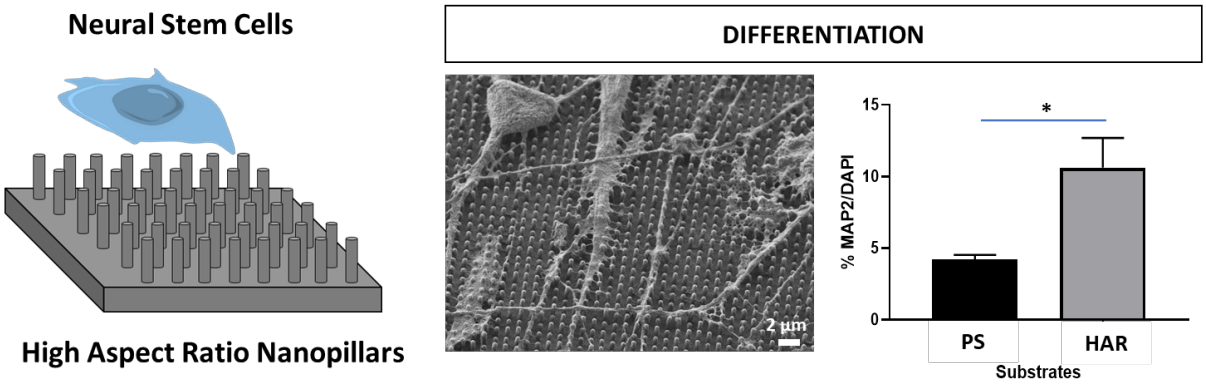
Topographical cues have emerged as promising tool for directing stem cells differentiation towards specific cell types, which provides an exciting opportunity to develop new cell therapeutic approaches [1]. However, there is still a need for better understanding of the relationship between mechanical stimulus provided by micro-nanotopography and the cell response obtained. To this aim, this work examines the behaviour and differentiation of an immortalized foetal human neural stem cell line (hNS1) [2] in response to the mechanical stimulus provided by a highly dense high-aspect-ratio (HAR) nanopillar topography. The HAR topography was created in polystyrene sheets by thermal nanoimprinting lithography (NIL).

It was found that the HAR topography reduced substantially the proliferation of the hNS1 cell when the cells were cultured in expansion conditions. Additionally, the hNS1 cells adopted a morphology of rounded cell bodies and aligned outgrowing protrusions to the topographic pattern. The HAR topography triggered the arrest of the cell cycle during the first stages of differentiation process and modulated cell cycle status, increasing the expression of p53 protein, involved in cell cycle control and fate decision control. After a longer-term differentiation period, an increased percentage of mature differentiated neurons were found on the HAR topography with cells extending their neurite-like projections along the topography lattice directions.

Our results suggest that the HAR topography modulate hNS1 behaviour in expansion conditions and increase the mature neuron population after longer term differentiation. Hence, HAR nanopillars emerge as a promising topography capable of influencing neural stem cells differentiation toward neuronal lineage with potential application in regenerative medicine and cell replacement therapies.



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Development of Quantum Dot platform to measure pathological proteins and pharmacological action in lymphoblasts from ALS patients

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Amyotrophic Lateral Sclerosis (ALS) is a motor neuron neurodegenerative disease, that leads to a progressive paralysis and death of the patients. TDP-43 is one of the key pathological signature in the disease which has important functions in the nucleus, such as stabilizing and transporting mRNA. In the disease, TDP-43 is aberrantly aggregated in the cytoplasm, phosphorylated by other proteins and the nucleo-cytoplasmic homeostasis is lost. There are not current treatments against ALS disease so it is an urgent biomedical need (1). Immortalized lymphoblasts from ALS patients recapitulate the disease and are here used to study the pathology, thus establishing a valuable cell line to study the mechanisms of the disease.

To perform the different assays, Quantum Dots (QDs) are used, which are luminescent nanoparticles with promising properties in this field since they present a versatile bioconjugation which allows to label many biomolecules of interest. These particles have a narrow emission spectrum, being several available which permit multiplexing applications (2).

First, we have developed a multiplexed assay to label different pathological proteins in these cells. Secondly, after studying their penetration properties in lymphoblasts, we determined that antibody-conjugated QDs are not able to entry the nucleus (3) of cells and thus could be used in flow cytometry to only target cytoplasmatic TDP-43. The objective is to implement a technique to determine quantitatively whether a reduction of TDP43 levels is observed in these cells after drug treatment, which would mean a recovery of nucleo-cytoplasmic protein transport, and a reduction of phosphorylated TDP43 protein levels after drug treatment.

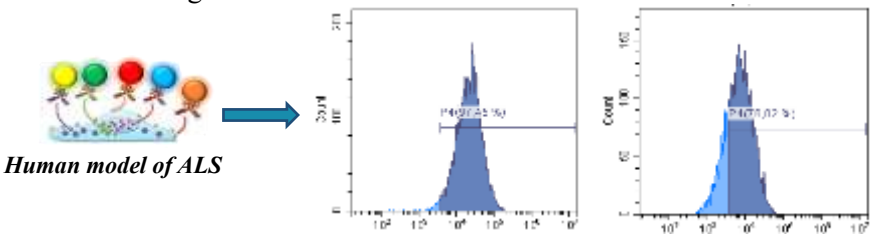

Figure 1. Analysis of TDP-43 and pTDP43 levels by flow cytometry through multiplexing technique in lymphoblast from ALS patients.

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BODIPY functionalized silicon oxide microparticles for intracellular glutathione sensing

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Biological thiols, including glutathione (GSH), play crucial roles in maintaining the appropriate redox status of biological systems. Molecular imaging based on fluorescent probes is a technique providing online fluorescence sensing of GSH[1]. However, they may have limitations such as achieving high cellular uptake or photobleaching. Self-assembled monolayers (SAMs) and microfabrication techniques related to the preparation of functional microparticles have been used to endow substrates with several properties, including a great control of size, shape, and better chemical functionalization[2]. SAMs are a key tool in the surface design of nanolayers for the bioactive coating of biomedical devices[3]. In this research, our main target is intracellular GSH sensing with micro–sensors. The advantage of using micro–sensors are long-term tracking and overcoming optical instability and biotoxicity. For this reason, a fluorescent GSH sensor, based on a BODIPY scaffold, was synthesized, characterized and immobilized on silicon oxide microparticles. The performance of functionalized microparticles in intracellular GSH sensing was evaluated using confocal microscopy.

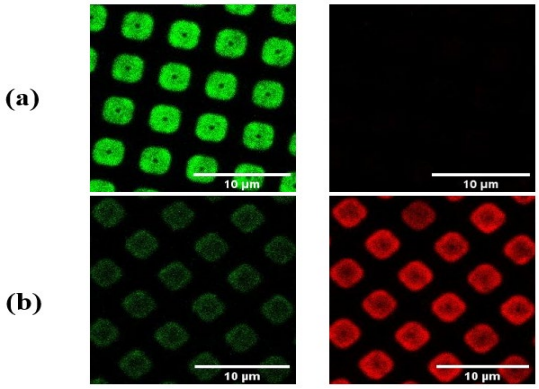

Figure 1. Confocal images of BODIPY functionalized silicon oxide microparticles (a) in the absence and (b) in the presence of GSH

Acknowledgment: This work was supported with funds from the Spanish Government Grant PID2020-115663GB-C32 funded by MCIN/AEI/10.13039/501100011033. S. B. thanks Generalitat de Catalunya for a predoctoral FISDUR scholarship.

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How nanobodies can improve current diagnosis tools and therapeutic approaches in lower respiratory tract infections.

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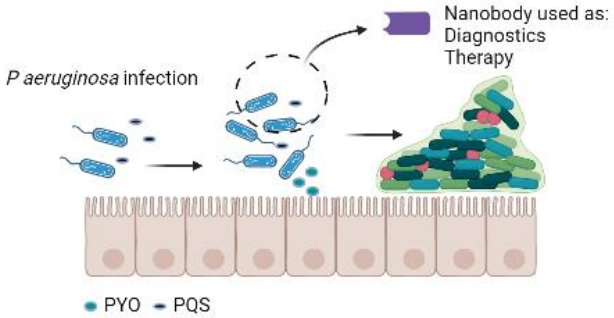
Nanobodies® (Nbs) have been appeared in the latest two decades as a serious alternative for the conventional antibodies for diagnostic and therapeutic applications. Nbs or VHH are the recombinant expression protein corresponding to the variable fraction from the heavy chain antibodies (HCAbs), characteristic IgG from camelids. Nbs have a molecular weight in the range of 12-15 kDa which is significally smaller that the conventional antibodies (Abs, 150kDa), but maintaining the biorecognition properties such as affinity, detectability, selectivity and reproducibility. According their highly structural stability, it confers an additional robustness and stability in front of temperature, organic solvents of these Nbs in respect to Abs. Also, due to the recombinant nature, Nbs can be expreseed in a cheap manner in bacterial culture being possible to produce in a gram scale/week. Moreover, the unique properties of Nbs are outlined like internalization, size, affinity, blood clearance, and labeling procedures make them as novel approaches to face some therapeutic applications. These outstanding properties made Nbs as ideal for the next generation of immunoassays for diagnosing purposes.
Thus, Nbs can be used for the improvement of the current features for the detection of infectious diseases and how can be used them in therapy to minimize or disrupt the cell-to-cell communication mechanism for *Pseudomonas aeruginosa* (PA) infectious. The mechanisms of communication between bacteria that controls their growth, biofilm formation and expression of virulence factors are commonly named *Quorum Sensing* (QS) and, basically, are governed by small molecules called autoinducers. In PA, one of the QS systems (Pqs system) is controlled by a certain quinolone molecule (PQS, Pseudomona quinolone signal molecule) which is specific from PA and also control a specific virulence factor called Pyocianin (PYO).
The group Nb4D group has a large experience in the detection and quantification of PQS[1] and PYO[2] in clinical samples using antibodies and also, use the same antibodies as novel therapeutic agent to protect mammalian cell cultures from the PYO action. All these experiences will be used for the evaluation of Nbs as promising tools to improve the antibodies features for the current PQS and PYO antibodies. To prove that, the same immunogens have been used to immunize alpaca with the final aim to obtain a cDNA library to select the most suitable Nb for PYO and PQS.



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New molecular assay based on nanotechnology for the HIV P24 early detection

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BACKGROUND/OBJETIVES:
Acute infection with human immunodeficiency virus (HIV) comprises 5 phases until seroconversion. Early HIV detection prevents viral spread, accelerating the antiretroviral therapy start. The most sensitive serological HIV diagnostic tests can detect 5.2.pg/ml HIV-1 P24 capsid protein, equivalent to around $5x10^4$ virions, only allowing diagnosis 3–4 weeks after infection. We present the first evaluation of a biosensor based on gold-plasmonic nanoparticles for the P24 detection in samples collected during early and chronic infection.
METHODS:
Plasmas from 23 patients in different HIV early infection phases (4 Eclipse/19 Fiebig I-V), 25 dried blood (DBS) samples with different HIV variants and viraemia, and 6 paired plasma/DBS from subjects in chronic infection were tested with a new plasmonic immunoassay, measuring by duplicate the plasmonic signal using AVAC scanner platform (Mecwins). The gold nanoparticles (GNPs) were optically identified, and the scattering of each GNP analyzed to characterize, classify and count the GNPs present on the silicon surface due to P24 detection with high specificity. Capture anti-P24-IBAB1 antibodies (Infinity) were used for silicon surface functionalization and detection-anti-P24-IBAB12-antibodies (Infinity) conjugated to carboxyl-polymer coated 100-nm-diameter GNPs (Nanopartz).
RESULTS:
The plasmonic molecular assay for P24 detection showed an extremely sensitivity for detecting HIV infection at early stages, undetectable with nucleic acid technologies (NAATs). The new biosensor could detect 75% samples in Eclipse Stage and all in Stage I. The rates of false negative samples increased in Stage II-V. P24 was detected in all but one samples in chronic infection. The signal detection for high vireaemia (>5log) samples was low, maybe due to antigen-antibodies complexes. Most HIV-1 variants were detected, presenting the same efficacy in DBS and plasma. The LOD of the new P24 assay was 10ag/mL (10^{-5}pg/mL), equivalent to one virion in 100µl plasma (10 virus/ml). This sensitivity is 4 orders of magnitude better than most sensitive immunoassay and 2 orders of magnitude better than NAATs.
CONCLUSIONS:
The new molecular nanotechnology detect HIV in samples from acute infection, even in the first week, before any commercial serological or molecular assay. The detection of ultra-low P24 levels could improve HIV early diagnosis to control AIDS pandemic. Further research is required to adapt AVAC technology for clinical routine testing at low cost and at the point-of-care.

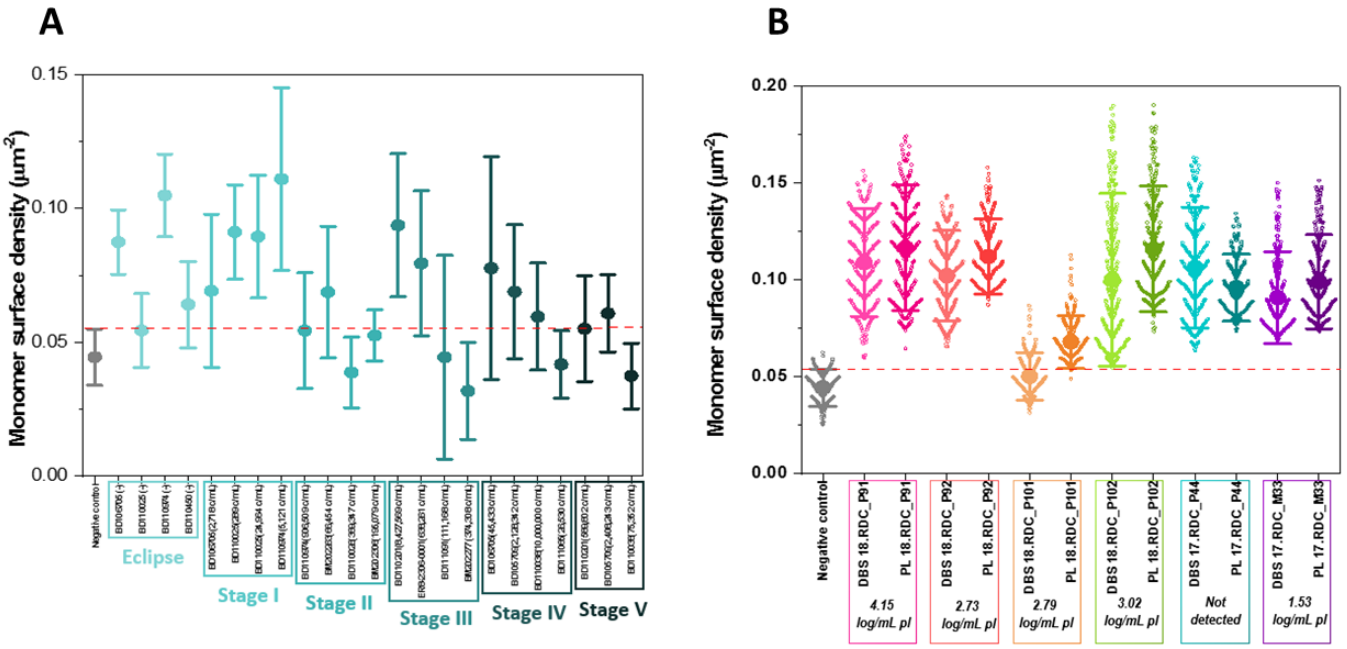

Figura 1. A) SERACARE panel results of recent infection. The chip detects 75% of Eclipse stage and 100% of Fiebig I stage samples. B) Clinical sample panel results comparing chip detection in paired DBS and plasma samples. There is no significant difference in detection between plasma and DBS samples or between the different viral loads.

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Characterization of aptamers for HIV-1 protease, integrase and p24 detection across HIV variants

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Background: Aptamers are nucleic acids with unique tertiary structure, high specificity and affinity for their targets, useful in diagnostic and therapeutic fields. Since HIV-1 presents high genetic variability, new diagnostic aptamer-based point-of-care (POC) devices detecting HIV proteins should recognize highly conserved domains to allow the molecular detection of all HIV variants. This study reports the selection, characterization and first evaluation of specific aptamers for recombinant HIV-1 protease (PR), integrase (IN) and P24 capsid proteins detection.
Methods: PR, IN and P24 sequences among HIV-1/HIV-2 variants were downloaded from Los Alamos Database (LANL). After aligning and translating into amino acids with MEGAv6.0, we identified the most conserved peptides using an in-house bioinformatics program (EpiMolBio). The most exposed and soluble were used for specific aptamers selection by SELEX. Affinity, sensitivity, and the best combination of aptamers for PR, IN or P24 detection were determined by ELONA (Enzyme-linked oligonucleotide assay) by triplicate using recombinant proteins. Data were analyzed with GraphPad-Prism. We also evaluated the cell-free viruses and plasma samples detection by anti-PR aptamers in ELONA.
Results: After analyzing all PR, IN and P24 LANL available sequences ascribed to multiple HIV variants, we identified 3 soluble, exposed and highly conserved peptides across variants in PR (GI peptide), IN (PE peptide) and P24 (PK peptide), showing ≥90% conservation across HIV-1 group M and HIV-2 sequences. The best 7 aptamers were characterized by ELONA. Aptamers GI6.1F and GI6.16F recognized GI with affinity of 10-15nM and sensitivity of 1,25-2,5pmol of PR. Aptamers PE5.1F, PE5.4F and PE6.8F recognized PE with affinity of 30-196nM and sensitivity of 0,38pmol of IN. Aptamers PK9.12F and PK9.14F recognized PK with affinity of 40-78nM and sensitivity of 0,26-0,52pmol of P24. We also identified the best aptamer pairs: GI6.1F+GI6.1F, PE5.4F+PE5.4F, PK9.14F+PK9.12F. GI6.1F+GI6.1F could detect HIV-1 cell-free viruses and plasma samples with at least 6 log of viremia (VL).
Conclusions: We present the first aptamers able to detect PR, IN or P24 conserved peptides within HIV variants, providing affinity, sensitivity, best combination in ELONA, defining the anti-PR aptamers able to detect cell-free viruses and plasma samples with high VL. New nanotechnology devices were required to obtain better sensitivity that allows earlier HIV detection.

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Benign and versatile synthesis of iron oxide nanoparticles and their study in biomedical applications.

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Ferrimagnetic iron oxide nanoparticles (IONPs) have been widely studied for biomedical applications as contrast agents in magnetic resonance imaging (MRI) and for magnetic hyperthermia. Their size, shape and composition, critical for these applications, are controlled using different synthesis routes[1]. Organic media synthesis and coprecipitation in water are the best known routes to obtain different shapes of nanoparticles with different properties. They involve the use of toxic reagents that need to be removed before using the particles in physiological media (e. g. organic solvents) or may cause safety problems during their industrial preparation (e. g. ammonia gases, strong bases).

The main aim of this work is to obtain IONPs with tunable controlled shapes, compositions and sizes that can be useful for hyperthermia therapy and MRI diagnosis, using non-expensive and environmentally friendly and non-toxic reagents.

A variation of the Massart[2] co-precipitation method using a non-toxic base is described in this work. The synthesis consists in a drop by drop sodium carbonate base addition to an iron chloride mixture solution. The procedure allows tuning the composition (from goethite to maghemite), the morphologies (elongated, spherical and cubic), and sizes from 16 to 28 nm (Fig. 1). These changes affect their MRI contrast enhancement properties and heating abilities and can be obtained by just changing the amount of base.

Characterization of the size, shape, composition, relaxation times for MRI and magnetic heating will be presented, as well as preliminary attempts to reduce the aggregation, improve the internalization in cells, and reduce the toxicity.

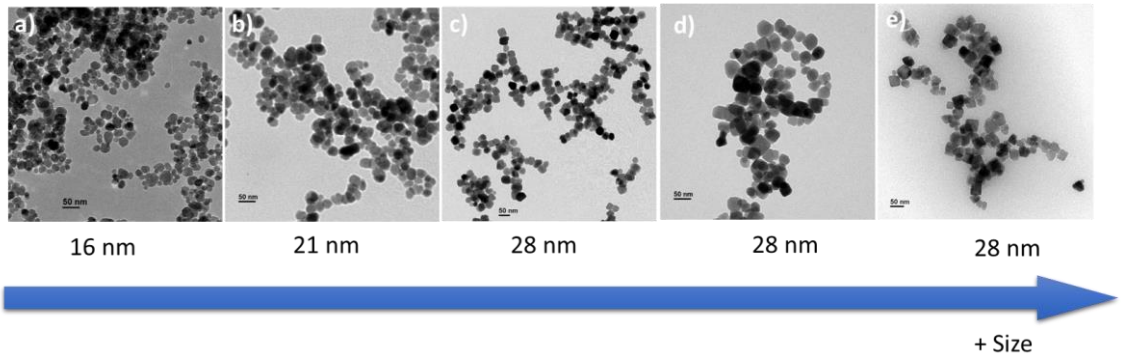

Figure 1.IONPs with different sizes and shapes obtained by slight changes of the method presented in this work. Scale bars = 50 nm.

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Fluorinated Smart Nanoprobes with Applications for Detection of MMP-2/9 after Stroke by ^{19}F MRI

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Magnetic resonance imaging (MRI) is a non-invasive imaging technique for diagnostic purposes. In particular, ^{19}F-MRI takes advantage of the lack of endogenous fluorine to produce images without interference from the background. This provides unambiguous detection suitable for quantification. To improve the information provided by this technique often a probe or contrast agent is used. Among the different types of contrast agents, smart OFF/ON probes are of particular interest as they are systems in which the OFF signal is switched to an ON state in response to an external stimulus. This selective stimulus is triggered, for example, by the presence or dysregulation of enzyme activity, which may act as of pathological processes. Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade all components of the extracellular matrix, and are involved in many of these processes. They produce deleterious effects during the early ischemic stage, but are beneficial in the recovery stage. We propose the synthesis smart probes based on gelatin nanoparticles (NPs) sensitive to matrix metalloproteinases (MMP-2/9) that encapsulate fluorine-labelled NPs detectable by ^{19}F MRI. We hypothesise that when the probe is encapsulated in gelatin NPs, the ^{19}F-MRI signal is turned OFF, but this signal is turned ON when disassembly of the gelatin NPs occurs, caused by digestion of the gelatin by MMPs (See figure 1). Optimisation of the synthesis and characterisation of gelatin and fluorinated NPs are key points for the design of a bioresponsive probe. On the one hand, gold NPs with an organic coating based on polyethylene glycol (PEG) ligands will be synthesised due to their solubility in water and good MR properties for ^{19}F-MRI. On the other hand, the two-step desolvation method using EDC/NHS as crosslinking agents will be used for gelatin synthesis. The characterisation of these NPs will be performed by transmission electron microscopy (TEM), Dynamic light scattering (DLS), inductively coupled plasma-mass spectrometry (ICP), etc. The design of these probes with OFF/ON characteristics shows the potential use of fluorinated NPs with applications in detection of MMP activation following stroke using ^{19}F-MRI.

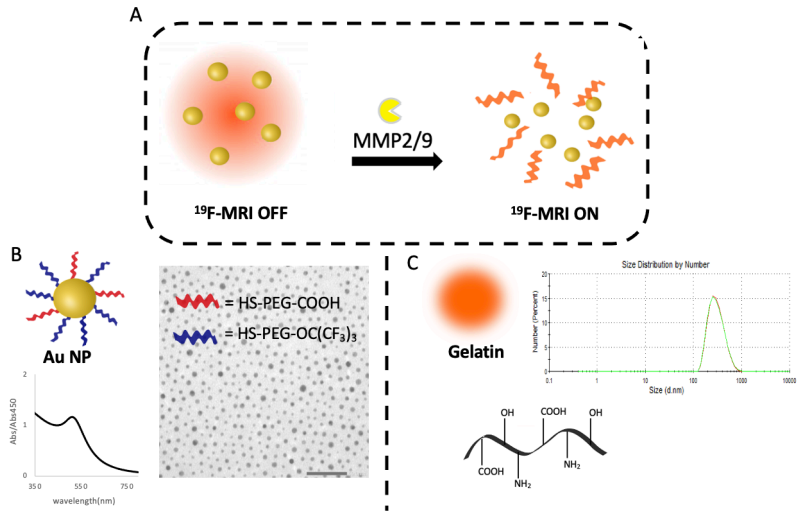

Figure 1. (A) Schematic representation of our OFF/ON smart probe where initially the 19F-MRI signal is turned OFF, but after digestion of the gelatine by the MMPs, the 19F-MRI signal is turned ON. (B) Above left is the gold NP with the carboxyl and fluorine ligands, on the right is the TEM of the NPs (x100), and below left is the UV-Vis of NPs with the characteristic plasmon at 520nm of Au-NPs. (C) Above left is the gelatin NP, on the right is the size distribution by number of gelatin NP, and below is a gelatine chain type A.

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IRON OXIDE NANOPARTICLES FOR T1 POSITIVE CONTRAST IN MAGNETIC RESONANCE IMAGING.

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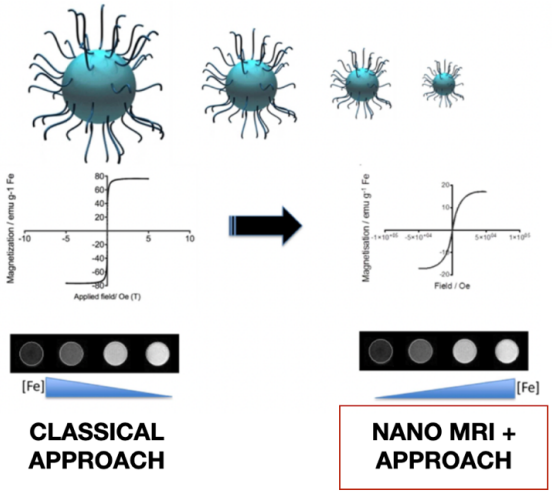
Iron oxide nanoparticles (IONP) are well-known owing to their magnetic properties and their potential as Magnetic Resonance Imaging (MRI) probes. For several years, IONP-products were clinically available, but industry discontinued the production of these nanoparticles due of the lack of a clear application. The main reason for this is their typical signal in MRI, due to their superparamagnetic behavior, they provide the so-called "negative" contrast, a darkening of the tissue there where the IONP accumulate. For many diseases, this type of signal is far from optimal for diagnosis. [1-3]

In the Nanomed+ project we are developing IONPs with the brightest possible signal without losing the biocompatibility and multifunctional character that these IONPs exhibit.

In this work we will present the best results obtained for the different approaches done. Synthetic routes as key change to produce IONPs for positive contrast in MRI and their application in the diagnosis of cardiovascular diseases.



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Functionalization of T-cells with microwave synthesized magnetic nanoparticles for their magnetic retention

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Adoptive T-cell transfer (ATC) therapy is a promising treatment against cancer, but it has a big drawback: the low proportion of T-cells that reach the tumour. Approaches that localize T-cells in target region are being studied to tackle this problem, such as those based on iron oxide magnetic nanoparticles (MNPs). This work evaluates if human and mouse T-cells, functionalized with innovative microwave (MW) synthesized MNPs, maintaining their biological integrity, could be retained by an external magnetic field (EMF).

MNPs of different sizes were obtained by microwave (MW)-assisted synthesis and coated with aminopropylsilane (APS). After physicochemical characterization, the biocompatibility between human (Jurkat) or mouse (primary $CD8^+$) T-cells and MNPs was analyzed by different experimental approaches: cell viability and biological integrity assays (flow cytometry), iron-association kinetics (ICP-OES) and MNPs localization in cells by microscopy (optical, confocal, electron). Magnetic T-cell retention was studied *in vitro* using a flow chamber system in presence of EMFs and functionality of CD8+ primary T-cells was analyzed. Finally, magnetic T-cell retention was studied *in vivo* in lymphoid organs.

All MW synthesized MNPs showed low toxicity and greater iron association to T-cells at 2-4 hours. MNPs treatment did not affect biological integrity of T-cells and different microscopy experiments showed that MNPs were present on cell membrane. Among different MW synthesized MNPs, APS coated 30 nm particles (MW30-APS) showed the highest magnetic retention capacity, similar to coprecipitation MNPs of around 10 nm (COP12-APS). Furthermore, CD8+ primary T-cell function was not affected after MNPs treatment, and their magnetic retention was increased in lymphoid organs when applying an EMF.

Microwave synthesized MNPs are biocompatible with human and mouse T-cells. Further, when applying an EMF, the highest magnetic T-cell retention was obtained with MW30-APS, similarly to COP12-APS. Magnetic T-cell retention was also increased after MNPs treatment *in vivo* when applying an EMF. Altogether, although microwave and coprecipitation synthesis methods showed similar results, microwave-assisted synthesis is a more suitable method to be used in clinical practice.

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Tumor solid stress reduces the nanomedicines penetration in tumors

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Assessing the transport properties of new nanomedicines across biological barriers is crucial for the design of nanoparticle-based effective therapies [1]. However, there is a lack of suitable *in vitro* platforms to evaluate the delivery efficacy of nanoparticles (NPs) to tumors. In this context, microfluidic devices have emerged as a promising tool to recreate *in vitro* the biophysical barriers present in the microenvironment around the tumor [2]. The delivery of NPs to the solid tumor, and ultimately their efficacy, is affected by the ability of the NPs to cross the physiological barriers and penetrate into the tumor. This process of NPs bio-distribution is dependent upon the NP's properties (size, shape, charge, chemical makeup) and also on the extracellular matrix (ECM) characteristics, which greatly affect the NP diffusive transport [3]. One important aspect to consider for NP delivery to solid tumors is the ECM densification as a consequence of the tumor overgrowth and pressure generated. How this solid stress affects the NPs penetration through the ECM in reaching the tumor is still unclear [4]. Here, to investigate this process, we present a novel PDMS-based microfluidic tumor-on-chip device capable of recreating the solid stress generated by growing tumors. This device employs a mobile membrane that exerts pressure on the surrounding matrix. This ECM is connected to a capillary that recreates a physiological blood vessel through which the NPs are delivered. Using this tumor-on-chip device, we have observed a reduction of up to 65% in the NPs permeability through Matrigel when pressure of 13 mbar was applied to the membrane. Our results indicate that the solid stress applied to the ECM greatly impacts the transport of NPs. Hence, tumor-on-chip devices may be useful predictive models for *in vitro* evaluation and optimization of the design parameters of nanomedicines for improved delivery to tumors.

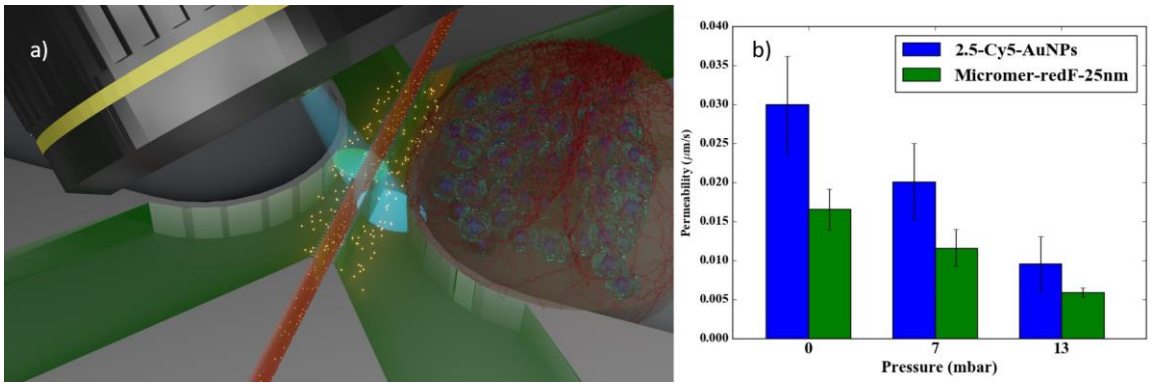

Figure 1: a) Recreation of the penetration process of nanoparticles in the device when the tumoral pressure is recreated using the PDMS membranes. b) Permeability coefficients of nanoparticles at different applied pressures.

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Preparation of Fe_3O_4/Au nanostructures for biomedical applications

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The preparation of superparamagnetic iron oxide nanoparticles (SPIONs) for potential biomedical applications, such as contrast agents for MRI, drug carriers or magnetic hyperthermia treatment, has been the subject of many research groups for the last decades. Nowadays, there is a growing interest in the combination of the magnetic properties and applications of SPIONs with other materials (oxides, metals) that provide additional capabilities for biomedicine.

In this work, we have synthesized hybrid materials of Fe_3O_4/Au by thermal decomposition according to a procedure similar to one reported by the Sun et al for SPIONs.[1] Before studying their potential applications in biomedicine, the hydrophobic hybrid nanostructures have been transferred to aqueous dispersion through a ligand substitution procedure.

The nanoparticles have been characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), vibrating sample magnetometry (VSM), Raman spectroscopy, time-domain nuclear magnetic resonance (TD-NMR) and magnetic heating under alternating magnetic fields.
We obtained nanohybrids with a gold core of ~8 nm and a shell of Fe_3O_4 of ~30 nm size. SERS properties have been studied using a Raman reporter (Figure 1).
Hence, these nanoparticles show potential for their use as magnetic hyperthermia agents as well as in SERS detection when employed cancer cell cultures.

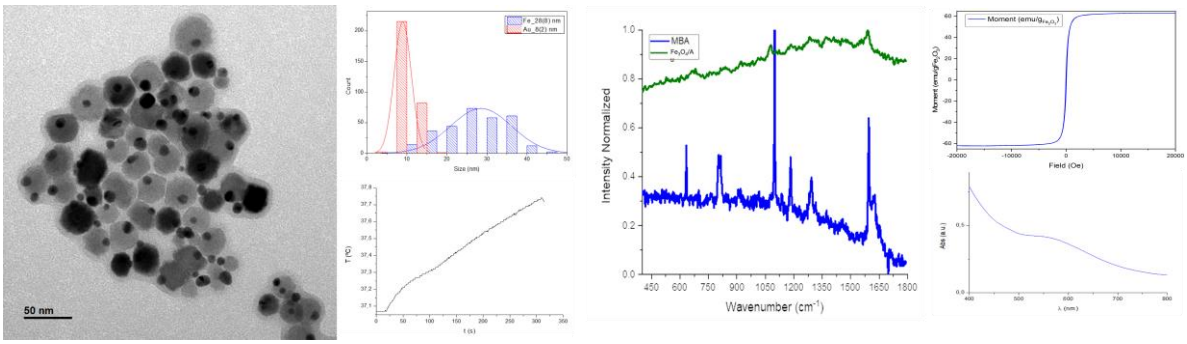

Figure 1. Fe_3O_4/Au characterization (from left to right and top to bottom): TEM, size histogram, temperature vs. time under alternating magnetic field, Raman spectra, magnetization by VSM and UV-Vis. spectrum.

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Biotransformed plasmonics of gold nanoparticles

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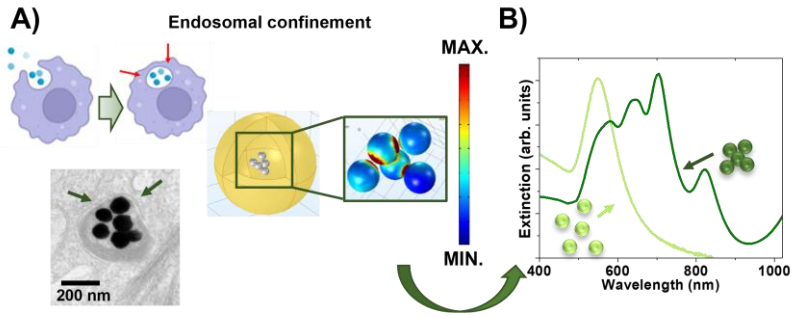
Cancer is a global health issue, leading one of the main causes of death every year. In 2020 more than 19.3 million cases were diagnosed. Furthermore, statistics predict an increase of cases of 47% for 2040. Henceforth, there is an urgent need to develop more effective and less invasive therapies. Within biomedical field, nanomedicine shows new possibilities and strategies proposing novel local therapies in the tumoral region. These promising therapies are mediated by nano-objects, highlighting noble metal nanoparticles (NPs). These NPs exhibit a characteristic property known as localized surface plasmon resonance (LSPR), which consists of an enhanced electric field that generates strong extinction of light.[1] This optical response depends on both intrinsic and extrinsic parameters of the nanomaterial. For example, size and composition of certain NPs can tune the plasmon response from the visible range to the near-infrared region (NIR) of the optical spectrum, making these nanomaterials suitable for biomedical applications. In fact, this resonant extinction results in a photothermal response which enables a controlled and localized heating (41-46 ºC) to promote selective induction of cancer cell death.[2]



Despite progress in this research field, there is currently a need to comprehend which extrinsic parameters can affect the optical response, how they modify this response and how they are affected by the intrinsic characteristics of NPs. Specifically, when these nanomaterials interact with biological media, LSPR can be modified as a result of the interplay between the NPs and the cellular environment (endosomal confinement in Figure A).[3] These modifications, and the consequent plasmonic coupling, alter the system efficiency and must be considered in the design and optimization of new nanotherapies. In this work, the photo-induced response of different gold metallic NPs has been explored in tumor cells, identifying the plasmonic coupling and consequent changes in the photothermal response. For that, we have designed a methodology for exploring experimental and theoretically the involved physical and therapeutic parameters. The experimental optical properties are validated with numerical modeling using COMSOL Multiphysics, considering different degrees of plasmonic coupling and their influence on the photothermal performance under NIR light exposure (Figure B).

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Fe/Au/Cu nanostructures for biomedical applications

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Magnetic nanoparticles (MNPs) have been extensively studied for biomedical applications during the last decades. They can be used as contrast agent in magnetic resonance imaging or as heating mediators in magnetic hyperthermia. More recently, magneto-plasmonic nanostructures have attracted interest because they combine the properties of MNPs with the possibility of being used in SERS imaging or sensing and in photothermia therapy.[1]

In this study, thermal decomposition syntheses based on *Sun et al.* have been developed to produce Fe/Au/Cu nanostructures.[2] Iron(III) acetylacetonate, gold(III) acetate, copper(II) acetylacetonate are used as reagents, and 1-octadecene as solvent. The nanostructures obtained are hydrophobic, so it is necessary a surface modification by hydrophilic ligands (DMSA) for their use in biomedicine.

Samples have been characterized (Fig. 1) by transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), X-ray diffraction (XRD), dynamic light scattering (DLS), Raman spectroscopy, time-domain nuclear magnetic resonance (TD-NMR), and magnetic heating measurements. The nanostructures are measured by TEM with a core size of 8 ± 2 nm and a shell size of 24 ± 4 nm. They show a superparamagnetic behaviour confirmed by VSM. They exhibit a hydrodynamic size of 63 nm in water (after surface modification), which indicates negligible or very low aggregation.

These Fe/Au/Cu nanoparticles combine present superparamagnetic behaviour and surface plasmon resonance in UV-Visible spectra. They are efficient in magnetic heating and display surface-enhance Raman spectroscopy (SERS), what could allow detecting the nanostructures in physiological media.

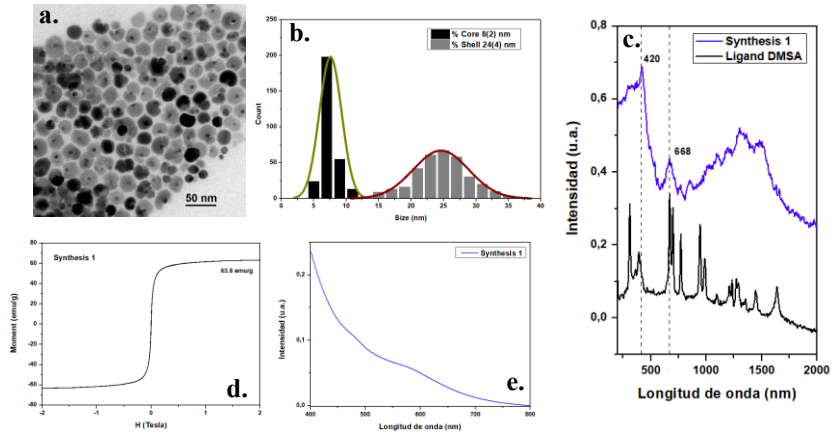

Figure 1: a. TEM Image 250X, **b.** TEM distribution size, **c.** Raman spectra, **d.** M(H) behaviour, and **e.** UV-Visible spectra.

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Rare-earth nanostructures as fluorescent nanothermometers and nanoheaters

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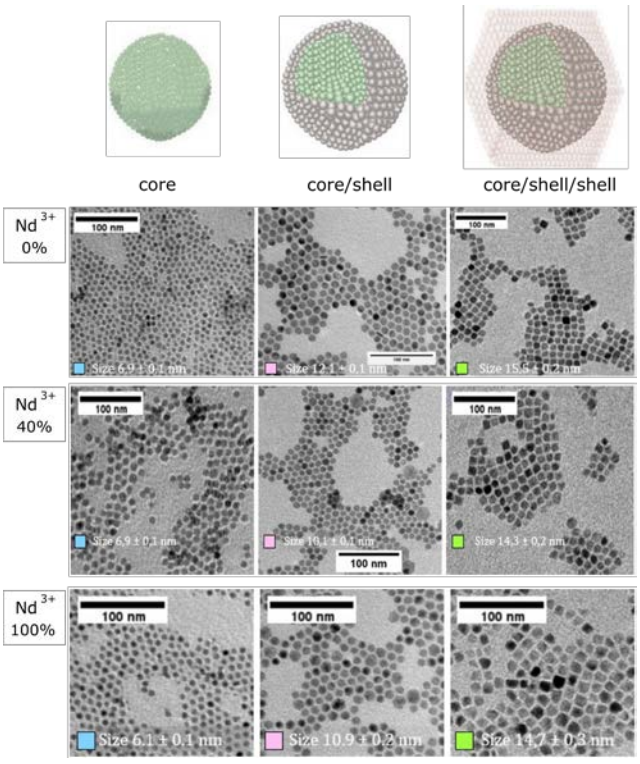
Rare-earth nanocrystals have recently proved as very efficient in vivo NIR luminescence lifetime nanothermometers [1]. The confining of the active ions, Nd^{3+} as sensitizers and Yb^{3+} as activators, in a thin intermediate shell inside the nanoparticle provides a remarkable thermal sensitivity. In addition, the viability of these core/shell/shell nanostructures for use in photothermal therapies has been shown very recently [2]. NIR-light to heat conversion is provided by the absorption and the non-radiative deexcitation of Nd^{3+} ions present in the active shell, whereas thermal sensing is achieved by simultaneously measuring the luminescence lifetime of the Yb^{3+} ions.

Our aim in this work is to improve the performance of these nanostructures as nanoheaters while maintaining the thermal sensitivity response. To do that, we doped the inert cores of the previous core/shell/shell nanoparticles with Nd^{3+} ions to increase the NIR-light to heat conversion. Thus, 7 nm core $NaYF_4$ nanoparticles doped with Nd^{3+} have been synthesized via a thermal decomposition method. Then, a 2 nm optically active shell, doped with 60% Nd^{3+} and 20% Yb^{3+} ions, has been grown around the core via a dropwise hot-injection method. Finally, these nanoparticles have been covered with a 1 nm inert shell of CaF_2. We changed the concentration of Nd^{3+} in the core to analyze the photo-thermal response of our nanostructures.



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This work was founded by the Comunidad de Madrid (S2017/BMD-3867 RENIM-CM), co-financed by the European Structural and Investment Fund and the Ministerio de Ciencia e Innovación (PID2021-122806OB-100, MAT2017-83111R and PID2019-106211RB-100) and by REACT PR38/21 ANTICIPA-UCM.

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Taming the influence of dipolar interactions in nanoparticle assemblies for magnetic hyperthermia

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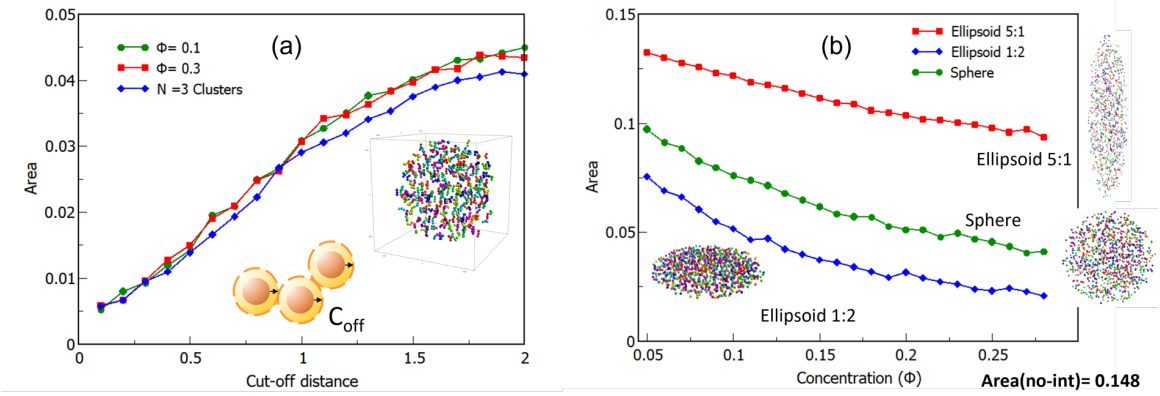
Magnetic hyperthermia is one of the most promising biomedical applications of magnetic nanoparticles (NP) and is intended to be alternative to cancer therapies based on drug delivery and radiotherapy. The range of suitable intrinsic parameters of the NP (such as saturation magnetization, anisotropy, shape and size) [1] and of applied magnetic ac fields that maximize the specific absorption rate (SAR) has been thoroughly studied [2] and mostly understood. However, there is still ongoing controversy on the role that aggregation state of the assemblies and dipolar interactions (DI) play on SAR. Here, we will study in regular lattices on SAR using Monte Carlo simulations of hysteresis loops in the macrospin approximation [3], showing that SAR can be increased or decreased with respect to the non-interacting case depending on the spatial arrangement of the NP assembly, particle size and separation. Next, we will show that, although in random assemblies dipolar interactions usually decrease SAR, their pernicious effect can be diminished by controlling: 1) the thickness of the surfactant covering the NP forming the clusters (Fig. a), 2) the regions on which the NP are deposited [4] (inside and at the surface of liposomes/cells, clusters), and 3) the global shape of the disordered assemblies (Fig. b). Acknowledgments: Work supported by Spanish MINECO (PGC2018-097789-B-I00, PID2019-109514RJ-I00), Catalan DURSI (2017SGR0598) and EU FEDER funds (Una manera de hacer Europa) also CSUC for supercomputer facilities.



Surfactant thickness (a) and concentration (b) dependence of the hysteresis loop area (SAR) for different kinds of NP random assemblies.

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Opto Electrical Nano-reactors for Wireless Cell Stimulation

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Throughout the years, various approaches using opto-electrical nanomaterials were carried out to treat diseases related to impairment of excitable cells. However, these approaches were limited to the usage of visible light which is restricted to superficial implants. Another limitation is the requirement of high light intensity leading to tissue damage due to excessive heating. Based on these facts, it is crucial to search alternative systems that are effective in deeper tissues without any harm.

In this study, we aim to develop opto-electric nanomaterials with high performance for wireless cells stimulation. The actuator device is based on Au/Si nanowires fabricated on silicon wafer with p-n junction for enhanced opto-electrical characteristics.

The electrical properties of the nanowires, i. e., photovoltage and photocurrent, were tested in phosphate buffer saline solution using two light sources corresponding to the first and second biological windows (810 nm and 1050 nm). Results showed no significant difference regarding the photovoltage obtained with nanowires compared to flat p-n Si wafer. However, photocurrent was substantially higher in both biological windows the nanowires sample than in the flat Si wafer. In addition, nanowires samples showed higher photocurrent at 810 nm than at 1050 nm. In order to translate the device performance at the cellular level, a human osteosarcoma Saos2 cell line was used, which expresses voltage gated calcium channels. First, we confirmed the biocompatibility of these nanowires through cell viability assays. Then, using the confocal laser scanning microscope, we recorded the opening of these channels in response to light illumination at 810 nm in both flat and Au/Si nanowires samples. At 450 µW light power, the nanowires system resulted in 41% of activated cells compared to 32% with flat wafer.

Our Au/Si nanowires device showed a combination of light induced capacitive and faradaic currents. Its biocompatibility and efficiency in exciting cells at very low light levels present them as a promising system for opto-electric actuation in deep tissues using NIR light. In the future, lower light intensity will be used and excitation efficiency at 1050 nm will be tested.

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Fabrication and characterization of BiTe-based NPs for Potential use as contrast agent in X-ray imaging

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We present the growth and characterization of nanoparticles (NPs) based on bismuth and tellurium to be used as contrast agents in X-ray imaging. These nanoparticles could be an alternative to the iodine-based contrast agents used today, since the NPs could generate more contrast due to the high atomic number of the elements that compose them. Ideally, NPs composed by elements with the highest atomic number are the best X-ray absorbers. The elements heavier than gold are: Mercury, Thallium, Lead, Bismuth and, then, with Polonium starts a series of radioactive elements. The materials selected for this application should be also stable, non-toxic and relatively cheap to reduce the cost of fabrication. Therefore, Mercury, Thallium, Lead and radioactive elements are to be excluded, leaving the Bismuth as the only realistic candidate.
Bismuth and Tellurium based nanoparticles have been fabricated for the first time using a gas-phase synthesis method called ion cluster source. This method provides great control over the size and chemical composition of the nanoparticles. The nanoparticles fabricated in this work have been characterized by atomic force microscopy (AFM), X-ray photoemission spectroscopy (XPS) and scanning transmission electron microscopy (STEM) to determine their size, fabrication rate, chemical composition, morphology and chemical structure. In order to compensate the difficulty of aggregation of bismuth and tellurium in the gas phase, we have used gold atoms as nucleation seeds. This approach has proved to be effective, synthesizing nanoparticles with up to 62% of bismuth telluride.